UNIVERSIDAD DE COSTA RICA

SISTEMA DE ESTUDIOS DE POSGRADO

TÍTULO: ESTACIONALIDAD, RECURSOS FLORALES Y CAMBIOS A LARGO PLAZO DE LA COMUNIDAD DE ABEJAS EUGLOSINAS DEL BOSQUE TROPICAL SECO DE COSTA RICA

Tesis sometida a la consideración de la Comisión del Programa de Estudios de Posgrado en Biología para optar al grado y título de Maestría Académica en Biología

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DEDICATORIA

A mi familia y amigos.

Y a todas las abejas (Incluso *Apis* que no me gusta tanto)

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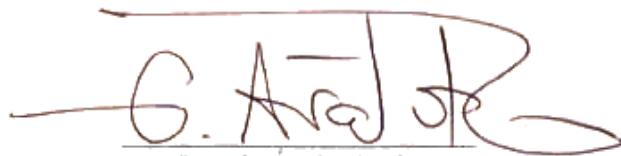
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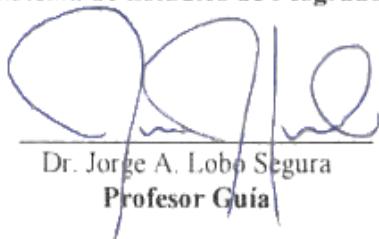
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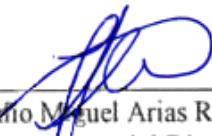
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RESUMEN

La potencial desaparición global de las abejas y su impacto en la economía y los ecosistemas naturales han atraído la atención de científicos, gobiernos y público en general. Sin embargo, aún faltan datos para respaldar el publicitado declive global de abejas, principalmente de especies de abejas fuera de las zonas templadas. Se ha determinado que el declive de abejas. Se ha determinado que el declive de abejas posee múltiples causas, la principal de ellas: la pérdida de hábitat. El bosque seco tropical (BTS) durante décadas sufrió una intensa deforestación. Pero en los últimos años gran parte del BTS de Costa Rica ha entrado en proceso de recuperación. Con esto en mente y utilizando los datos de diversidad de abejas orquídeas generados por Janzen *et al*. (1982) en el BTS del Parque Nacional Santa Rosa en el noroeste del país, planteamos una pregunta general: ¿Qué cambios han ocurrido en la diversidad, estacionalidad y composición de la comunidad de abejas euglossinas en el bosque tropical seco durante los últimos 40 años y ¿Cómo se relacionan estos cambios con la recuperación actual del bosque seco de Costa Rica? Usamos los mismos métodos: muestreo en la mitad de la estación seca, al comienzo, a la mitad y al final de la estación lluviosa, usando los mismos cinco atrayentes químicos. No observamos diferencias significativas en el número efectivo de especies entre bosques de 1977, 2018 y 2019. Esto a pesar de que no encontramos cuatro especies de abejas reportadas hace 40 años en el anteriormente en el TDF de Costa Rica. Además observamos aparentes cambios en la estructura de la comunidad, siendo la composición de especies de los fragmentos de bosque de 1977 más similares a la composición observada actualmente en pastos. También notamos un cambio estacional en la abundancia máxima de algunas especies de abejas euglosinas en TDF, como también se señaló hace 40 años. Además observamos que hay especies como *Euglossa dilemma* que habitan en pastos. Es posible que ciertos rasgos que posee esta especie le permita utilizar los recursos disponibles en pastos. Y aunque esta especie no presentó diferencias en el tamaño promedio, observamos que la variación en el tamaño del cuerpo de esta especie disminuye en pastos. Podemos concluir que la regeneración de BTS ha permitido que la diversidad de abejas euglosinas se mantenga más o menos estable a lo largo de estos años, contrarrestando los efectos negativos de otros posibles factores de declive de las abejas.

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FIRMA ESTUDIANTE

INTRODUCCIÓN GENERAL

Desde los años 90, el posible declive global de abejas ha sido el centro de atención de científicos y público en general (Ghazoul, 2005, Potts *et al*. 2010). La reducción en las poblaciones de abejas en el mundo podría tener un efecto importante sobre la seguridad alimentaria y la polinización de las plantas en general, ya que las abejas brindan un servicio ecosistémico fundamental, la polinización de la mayor cantidad de especies de plantas (Michener, 2000, Kremen *et al*., 2002; Ricketts *et al.*, 2008). Se ha estimado un valor económico de la polinización por insectos en 153 billones de Euros, lo cual equivale a 9.5% de la producción agrícola mundial (Gallai *et al*., 2009). Específicamente, las abejas son las encargadas de garantizar la reproducción sexual de la mayoría de plantas silvestres y mejorar la producción del 70% de los cultivos utilizados para la alimentación humana en el trópico (Roubick, 1995) y el 84% en Europa (Williams, 1994).

La escaza evidencia del declive se basa en la abeja de la miel (*Apis mellifera*) (Potts *et al*., 2010b, Ellis *et al*., 2010; Kulhanek *et al*. 2017) y abejorros (*Bombus* spp) (Kosior *et al*., 2007; Grixti *et al*., 2009; Biesmeijer *et al*., 2006), principalmente de climas templados, las cuales representan menos del 1% de la diversidad total (Ghazoul, 2015). Algunos estudios en Europa han incluido otras especies de abejas, las cuales muestran o no una disminución en sus poblaciones (Biesmeijer *et al*., 2006; Winfree *et al*. 2009; Ollerton *et al*., 2014; Hofmann *et al*., 2018; van Dooren, 2019). A pesar de ser altamente diverso, en el Neotrópico se han realizado pocos estudios relacionados con el declive de abejas. La mayoría de estos estudios muestran una clara disminución no solo en la riqueza de especies sino también en su abundancia (Frankie *et al*., 2009; Martins *et al*., 2013, Cardoso y Gonçalves, 2018; Vega-Hidalgo *et al*., 2020), aunque otros no muestran ninguna tendencia a la baja (Roubik y Ackerman, 1987; Roubik, 2001; Nemésio *et al*., 2015). Los estudios mencionados anteriormente son estudios a largo plazo que pueden reflejar procesos que en otras escalas de tiempo no pueden observarse, sin embargo, son laboriosos, costosos y logísticamente complicados (Armesto, 1990).

Causas del declive de las poblaciones de abejas

Se ha señalado que el declive de las abejas tiene causas múltiples, entre ellas: los pesticidas, los patógenos, la dieta deficiente, la introducción de especies exóticas y cambio

climático (Williams *et al*., 2010; Goulson *et al*. 2015). No obstante, se ha señalado a la pérdida de hábitat como la principal causa del declive en la diversidad de abejas (Kremen *et al*., 2002; Winfree *et al*., 2009; Williams *et al*., 2010). La pérdida del hábitat es causada principalmente por la expansión agrícola y urbana, que provoca la homogenización del paisaje y por ende una menor disponibilidad de sitios para la anidación y forrajeo de las abejas (Kremen *et al*., 2002; Winfree *et al*., 2009).

Generalmente se ha considerado que la disminución de la cobertura boscosa deteriora las condiciones del hábitat para las abejas (Powell y Powell, 1987; Aizen y Feisinger, 2002; Taki *et al*., 2007). Aunque se han obtenido resultados contrarios, donde sitios con cierto grado de alteración antropológica pueden albergar un mayor número de especies e individuos en comparación con bosques (Winfree *et al*., 2007). Por lo cual, se ha concluido que la historia natural de cada especie es la que moldea la respuesta a la pérdida del bosque y por lo tanto es difícil observar patrones claros (Powell y Powell, 1987, Cane 2001, Moreira *et al*., 2017).

El bosque tropical seco en Costa Rica

El bosque seco, es uno de los tipos de vegetación mayormente amenazado y la mayoría no se encuentra bajo ninguna categoría de protección (Janzen, 1986; Gerhardt 1993; Olson, 2000). La distribución de este tipo de vegetación es restringida, la mayor parte concentrada en Suramérica y una menor área en las Antillas y Mesoamérica (Miles *et al*. 2006). Se ha observado que las principales amenazas a las que se encuentra sujeto el bosque seco son: el cambio climático, el aislamiento de fragmentos, la presión de la agricultura, el fuego, el aumento de la población humana (Olson, 2000; Miles *et al*., 2006).

En Costa Rica, el bosque tropical seco, o bosque deciduo, se concentra principalmente en la provincia de Guanacaste, y durante décadas (1950-1990) experimentó una intensa conversión a pastizales para la producción de ganado vacuno (Arroyo-Mora *et al*., 2005, Calvo *et al*., 2009). Sin embargo, a partir de los años 90s aumenta drásticamente la reforestación hasta convertirse en la provincia con la tasa más alta de reforestación para el 2005 (Tapia, 2016). La caída de los precios de la carne, el crecimiento de la industria turística y una transformación estructural de la sociedad, y no un cambio en las políticas de conservación, parecen ser los responsables del aumento de la reforestación en Guanacaste (Calvo *et al*., 2009). Sin embargo, la creación de áreas protegidas, han tenido un papel

fundamental en el aumento de la cobertura boscosa de la región. Desde la creación Parque Nacional Santa Rosa en 1971, con tan sólo 10 000 ha, el Área de Conservación Guanacaste ha aumentado su área y en la actualidad comprende 163 000 ha (Arroyo *et al*. 2005; Pringle, 2017). Además, el ACG ha trabajado en la integración de la población para la protección de la biodiversidad, al fomentar la educación ambiental y promoción del conocimiento de la diversidad biológica del área protegida (Janzen, 2000).

   Abejas de las orquídeas y la pérdida de hábitat

   Las abejas euglossinas, también conocidas como abejas de las orquídeas, poseen una distribución natural restringida al Neotrópico (Roubick, 2001; Nemésio, 2009). Estas abejas visitan miles de plantas para la recolección ya sea de polen, néctar, resina y/o sustancias aromáticas, con lo cual garantizan la polinización de las mismas (Roubick y Hanson, 2004). La recolección de fragancias por parte de los machos euglossinos, es una de las adaptaciones más particulares de este grupo, y la cual promueve la polinización de aproximadamente el 10% de las orquídeas neotropicales (Ramírez *et al*. 2011). Además, contrario a otros grupos de abejas, las euglossinas polinizan plantas que presentan poblaciones poco densas y ampliamente dispersas (Janzen, 1971; Brosi *et al*., 2008).

   Se ha señalado que la pérdida de hábitat tiene un efecto negativo sobre las poblaciones estas abejas, el cual puede ser fuerte (Brosi *et al*., 2007), leve (Tonhasca *et al*. 2002), o dependiente de la historia natural de cada especie (Moreira *et al*., 2017). Además, comparadas con otros grupos de insectos, estas abejas presentan poblaciones con una alta estabilidad a través del tiempo (Roubik y Ackerman, 1987; Roubik, 2001; Nemésio *et al*. 2015). Sin embargo, recientemente Vega-Hidalgo *et al*. (2020) han demostrado que incluso en áreas sin presiones humanas evidentes este grupo de abejas muestra una fuerte disminución en sus poblaciones. De ahí la importancia de considerar rasgos funcionales de las especies para obtener una mejor noción en cómo estas abejas responden a la pérdida de hábitat.

   Rasgos funcionales en respuesta a la pérdida de hábitat

   Un rasgo funcional es toda aquella característica morfológica, estructural, funcional, bioquímica o de comportamiento que influye en la adaptabilidad de un organismo (McGill *et al*. 2006; Violle *et al*. 2007). Cada rasgo funcional varía espacial y temporalmente ante el cambio en las condiciones ambientales (Violle *et al*., 2007), tanto dentro como entre

especies. En insectos sociales, como las abejas, la variación inter-específica de rasgos como la socialidad, especialización en la dieta, sustrato de anidación y el tamaño del cuerpo han sido estudiados para entender la respuesta ante el cambio en el uso del paisaje (Biesmeijer *et al*., 2006; Moretti *et al*., 2009). Sin embargo, la variación intra-específica en respuesta a esos cambios ha sido menos estudiada (Warzecha *et al*., 2016).

El tamaño del cuerpo de los insectos es un rasgo con alta plasticidad y uno de los más estudiados, ya que es afectado por factores ambientales y genéticos (Honěk, 1993, Davidowitz et al., 2003). La temperatura del ambiente y la cantidad y/o calidad de recursos para la dieta durante la fase larval, son dos de las causas próximas que explican la variación en el tamaño del cuerpo de una especie (Bosch y Vicens 2002; Davidowitz *et al*., 2003). El tamaño del cuerpo de las abejas se encuentra relacionado con rasgos como; el rango de vuelo (Greenleaf *et al*., 2007), periodos de forrajeo (Willmer y Finlayson, 2014), respuesta inmune (Arriaga-Osnaya *et al*., 2017), fecundidad (Honěk, 1993), especialización de la dieta (Bommarco *et al*., 2010), entre otras.

Para contribuir a la comprensión del efecto de la pérdida de hábitat en la comunidad de abejas euglosinas tanto en la diversidad y estructura de la comunidad, así como la variación en los rasgos funcionales como el tamaño del cuerpo, planteamos lo siguiente:

Objetivo General

Determinar el efecto de la pérdida/ganancia de hábitat en la diversidad, composición, patrones estacionales y el tamaño del cuerpo de la comunidad de abejas euglosinas, y su relación con la recuperación actual del bosque seco de Costa Rica.

Objetivos específicos

1. Describir los potenciales cambios han ocurrido en la diversidad (riqueza y abundancia), estacionalidad y composición de la comunidad de abejas euglossinas en el bosque tropical seco durante los últimos 40 años, y su relación la actual recuperación del bosque seco de Costa Rica.

2. Determinar si existen diferencias en la diversidad, estacionalidad y composición de las abejas orquídeas entre bosques y sitios dominados por pastos.

3. Determinar si hay diferencias en el promedio y magnitud de variación del tamaño del cuerpo de *E. dilemma* en hábitats de bosques y pastos.

4. Determinar si la estación del año afecta el tamaño del cuerpo y abundancia de *E. dilemma* en bosques y pastos.

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CAPÍTULO 1

Formato para la revista: -

Comparison of the orchid bee community in a dry tropical forest of Costa Rica after 40 years

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Abstract

Global bee decline and its impact on the pollination in agricultural and natural ecosystems have attracted public attention. However, more data is needed to show their generality and intensity around different ecosystems, especially in tropics. The main cause of bee decline is habitat loss. Tropical dry forest (TDF) for decades underwent intense deforestation. But in recently, a large part of the TDF of Costa Rica has entered a recovery process. Using diversity data of orchid bees generated by Janzen *et al*. (1982) on TDF of Guanacaste Conservation Area in the northwest of the country, we posed a general question: What changes have occurred in diversity, composition, and seasonality of the euglossine bee community in the TDF during the last 40 years and how are these changes related to the current recovery of this forest in Costa Rica. We perform a sampling of euglossine bees during 2018-2019, using the same methods applied previously. In order to characterize the response of euglossine bee to habitat loss, we extended the sampling to adjacent pastures to protected area. With the loss (4) and gain (3) of species, we did not find significant differences in species richness between years were observed. The composition of the euglossine bee community in the protected area in 1977 is more similar to present population in pastures than to the actual populations in forests, where the presence of forest-dependent species has been favored. The pattern of seasonal changes in the peak abundance of euglossine bees during the year, as noted 40 years ago, was also observed in the present populations. We can conclude that the regeneration of TDF has allowed the diversity of euglossine bees to remain stable throughout these years, counteracting the negative effects of other possible factors of bee decline.

Key words: Bee decline, habitat loss, species richness, land-use change, euglossine.

INTRODUCTION

Lately, alarming news about the global bee disappearance has attracted the attention of governments, the private sector, and the general public (Wilson *et al*., 2017). This concern is based on the role that bees play as the main pollinating taxon of many crops, and therefore, the huge economic impact that the loss of these organisms would have (Michener, 2007; Kremen *et al*., 2002; Klein *et al*., 2007; Ricketts *et al*., 2008; Gallai *et al*., 2009). While this discussion has stimulated more research and initiatives for bee protection (Ghazoul, 2005), the loss of diversity in any taxa should be in the public interest and should not be proportional to its economic value.

Although several studies have shown a reduction in populations of bees, the evidence is concentrated in honey bees (*Apis mellifera*) (Ellis *et al*., 2010; Potts *et al*., 2010: Kulhanek *et al*. 2017) and bumblebees (*Bombus* spp) (Kosior *et al*., 2007; Grixti *et al*., 2009; Jacobson *et al*., 2017). Some studies in Europe have included other bee species, and do or do not show bee decline (Biesmeijer *et al*., 2006; Winfree *et al*. 2009; Ollerton *et al*., 2014; Hofmann *et al*., 2018; Dooren, 2019). The tropics have been poorly studied, despite their highly diverse ecosystems. The few studies done in this region show a apparent decrease not only in the richness of bee species but also in their abundance (Frankie *et al*., 2009; Martins *et al*., 2013, Vega-Hidalgo, 2020), although others do not show any trend toward declining (Roubik and Ackerman, 1987; Roubik, 2001; Nemésio *et al*., 2015). The studies mentioned above are long-term (multi-year) studies that can reflect processes that at other time scales could not be observed. However, they are laborious, expensive, and logistically complicated (Armesto, 1990, Lindenmayer *et al*. 2012).

It has been pointed out that bee decline has multiple drivers such as pesticides, pathogens, global warming, etc. (Williams *et al*., 2010; Goulson *et al*. 2015). Despite this, habitat loss, associated with the accelerated decrease in forest cover, has been identified as the main cause of bee decline (Kremen *et al*., 2002; Winfree *et al*., 2009, Sánchez-Bayo and Wyckhuys, 2019). Due to deforestation, the tropical deciduous dry forest is among the most endangered vegetation types on the planet (Janzen, 1988; Gerhardt, 1993; Miles *et al*. 2006). In Costa Rica, the tropical dry forest is concentrated mainly in Guanacaste province, and for decades (1950-1995) underwent intense conversion to pastures for livestock production (Arroyo-Mora *et al*., 2005; Calvo *et al*., 2009). But from 2000 to 2015 this region

has experimented the highest reforestation rate in the country (Calvo *et al*., 2009; Tapia, 2016, Fig. 1). During the period of greatest deforestation, Janzen *et al.* (1982) monitored seasonal changes in male euglossine bees diversity between 1977 and 1979 in Santa Rosa National Park (SRNP), a protected in Guanacaste. The methodology of that study (based on bait fragrances) makes it highly replicable, and a comparison of current euglossine bee diversity with that observed in the past may help to understand the effect of habitat change on the bee community.

Euglossine bees (Apidae: Apinae), also known as orchid bees, are distributed in the Neotropics (Nemésio, 2009) and their response to habitat loss has been variable (Moreira *et al*., 2017). In some cases a negative effect of habitat loss on diversity and changes in phenology has been found (Powell and Powell, 1987; Roubik and Ackerman, 1987; Cândido *et al*., 2018). But because some of this species are capable of using fragmented habitats, have been characterized as transitional habitat bees (Brosi, 2009; Tonhasca *et al*., 2009). For this, it is necessary to be careful with generalizations about the response of an insect group to environmental changes, since it seems to be dependent on their life history (Cane *et al*., 2001; Moreira *et al*., 2017). Likewise, habitat loss co-occurs with other factors such as pollution and climate change, and therefore its effects on bee diversity are not easily defined. For this reason more studies are necessary to measure and predict the impact of habitat loss on euglossine bee diversity (Goulson *et al*., 2015).

To contribute to an understanding of the effect of habitat loss on euglossine populations, we addressed the following questions: i) What changes have occurred in the diversity (richness and abundance), seasonality and composition of the euglossine bee community in the dry tropical forest during the last 40 years?, ii) How are these changes related to the current recovery of the dry forest of Costa Rica? To answer these questions, we replicated the sampling carried out by Janzen *et al.* (1982) in SRNP. Because 40 years ago these sites were dominated by pastures, we included in the current sample disturbed areas outside PNSR, with which we addressed the following questions: iii) Are differences in orchid bee diversity, seasonality, and/or composition between sites related to forest cover (forest vs. pastures)?, and iv) Is the bee community of sites within current pastures similar to the community observed 40 years ago? As the habitat loss is one of the main drivers of bee decline(references above), we expect that by increasing forest cover in the region, bee

diversity will be maintained or increased. We expected to observe differences in the diversity, seasonality, and composition of the bee community in current forests compared with that observed 40 years ago, due to changes in vegetation that occurred over the years. In addition, since SRNP was a region dominated by pastures in the past, we expected a greater similarity between the communities of euglossine bees in 1977 with that observed in current pastures.

MATERIALS AND METHODS

Study site- We carried out this study during the years 2018 and 2019 in the Santa Rosa Sector (known as SRNP in1977) in the Área de Conservación Guanacaste (ACG) and adjacent grasslands, in the northwest of the province of Guanacaste, Costa Rica (10° 45' - 11° 00' N, 85° 30' - 85° 45' W, Fig. 2), on the SRNP plateau at 300 m altitude. SRNP comprises two types of forest: the evergreen forest dominated by oak trees (*Quercus oleoides*) and tropical dry forest (TDF) (Hartshorn, 1983). The TDF of SRNP has an average annual temperature range of 26.6 to 27.5 ° C, and receives an average rainfall of 1390-1800 mm annually, with a high degree of year-to-year variation (Gillespie *et al*., 2000; Janzen, 2000; Magnani, 2018). The TDF is characterized by a marked seasonality, with 6 dry months ranging from late December to mid-June, a period in which most woody plants lose their leaves (Janzen, 1993; Gillespie *et al*., 2000). In mature TDF it is common to find trees like madroño (*Calycophyllum candidissimum*), madero negro (*Gliricidia sepium*), ojoche (*Brosimum alicastrum*), guácimo macho (*Luehea speciosa*), chicle (*Manilkara chicle*), chaperno (*Lonchocarpus minimiflorus*), and manteco (*Trichilia martiana*) (Powers *et al*., 2009).

Sampling sites- We selected the three sampling sites from the study previously carried out by Janzen *et al*. (1982) that corresponded to fragments of TDF located at 300 m altitude in SRNP. The first site is a deciduous regenerating secondary forest, the second is a semi-evergreen forest and the third is an oak forest (Janzen *et al*. 1982). During 2018, we sampled during two consecutive days in each site in each sample period to take into the account the daily variation in the same season. We did not observe significant changes in species richness and abundance between days at the same site, so for the statistical analysis we used only the data of the first day of sampling for each site. For grassland censuses, we

selected three open grass-dominated sites. The first site is located 5.5 km from the limits of SRNP, near the Inter-American highway intersection with Quebrada Grande, the second site is in Las Melinas neighborhood in Cuajiniquil 2.8 km from SRNP, and the third one at Hacienda Rosa María 1.5 km south of the Casona de Santa Rosa, approximately 500 m away from the protected area (Fig. 2). These pastures were at approximately the same altitude as the forest sites and we used the same collection periods and methods.

Bee samples- We used the male population as representatives of the general population of euglossine bees. Males are easy to sample because they are fragrance collectors, which can be used to attract them to specific sites. Also, males have more pronounced morphological characters, which facilitate their identification (Roubik and Hanson, 2004). We sampled from 7 to 11 a.m. during the same sampling periods as in the previous study (Janzen *et al*., 1982): in the middle of the dry season (March), beginning, middle, and end of the rainy season (June, August, and December, respectively). We also used the same five chemical attractants: cineol, eugenol, methyl cinnamate (solid), benzyl acetate, and methyl salicylate, 99% pure from Sigma-Aldrich brand. We moistened cotton balls of approximately 5 cm diameter with 3 ml of each attractant and placed them 1.5 m above the ground hanging from branches of trees or shrubs. In the case of pastures, we used living fence to hang cotton. Instead of collecting all the bees attracted to the fragrances, as was done in the previous study, we did not collect the bees that we could identify to species level in the field. In the field we used a 40x-25mm magnifying glass to identify the species and release them. However, due to the difficulty of accurately assessing some characters, some species mainly of the genus *Euglossa* (for example, *Euglossa variabilis* and *Euglossa townsendi*), were mostly captured to avoid confusing them with other possible species. To avoid resampling of the same individual after identification and releasing, we marked bees on the wing with a permanent fine-tip marker, with different colors for each sampling site. In this way, any recapture in the same period is detectable. For bee identification, we use the key of "Orchid bees of Tropical America" (Roubik and Hanson, 2004). The most important taxonomic change is the species previously reported as *Euglossa viridissima* is currently recognized as *Euglossa dilemma* in Costa Rica (Eltz *et al*., 2011). We deposited the collected bees for reference in the Zoology Museum of the University of Costa Rica (Museo de Zoología, MZUCR).

Statistical analysis: We tabulated the abundance of each species by season for each sampling site and year. For the preparation of statistical tests and comparative graphs, we used the R program (R Core Team, 2019) and specific packages that are mentioned in each analysis. To compare the alpha diversity of current euglossine bee community (2018-2019) at forest and grassland sites with the diversity observed 40 years ago, we calculated rarefaction curves with the first three orders of Hill's numbers (q) (Hill, 1973), where, the sensitivity to the abundance of the species increases as q parameter increases. So, $q = 0$ indicates the accumulated species richness, in which the abundance of individuals doesn't matter; $q = 1$, effective number of typical species, with each species having a weight proportional to its abundance, and $q = 2$, the effective number of dominant species, which gives more weight to the abundant species than the rare ones (Chao *et al*., 2014). For this analysis, we used the *iNEXT* package (Hsieh *et al*., 2016). To compare the structure of the current euglossine bee community (in forests and pastures) and that observed in the previous study, and seasonal effects on these communities, we carried out a Multivariate Analysis of Variance with permutations (ADONIS) using the Bray-Curtis distance *vegan* package (Oksanen *et al*., 2017). We included the effect of the season in that analysis. We use a Non-Metric Multidimensional Scaling analysis (NMDS) using the isoMDS function from the *MASS* package (Venables *et al*. 2002) to graph the result. We also used an indicator species analysis (ISA) from the *indicspecies* package (De Caceres and Legendre, 2009), to determine which bee species might be good indicators for forests and pastures. We use the species with more than 50 individuals in current forests and pastures to observe the effect of habitat and season on individual fluctuations using a generalized linear model with a Poisson distribution.

RESULTS

We observed a slight decrease in species richness, 15 species in 2018, and 14 in 2019, compared to 18 species in 1977, reported by Janzen *et al*. (1982) (Appendix: Table A1). In 2018, we found a greater general abundance, 1019 individuals, followed in 2019 with 907, while in 1977, they collected 720 individualsin the same sampling sites. On the other hand, compared to those forest sites, we found a smaller number of individuals and species of euglossine bees in pastures. In 2018, we observed 502 bees belonging to 10

species in pastures, while in 2019 we observed 291 individuals distributed in 9 species. The 4 genera names will be abbreviated as follows: *Euglossa=Eg.*, *Eulaema=El.*, *Exaerete= Ex.* and *Eufriesea=Ef.*

In all the sites and years analyzed, we estimated that 99% of the community diversity was detected (Fig. 3). Using species rarefaction curves and their calculated standard deviation, the species richness of euglossine bees (q = 0) for 1977 does not differ from the found in 2018 and 2019 forests, but it is greater than that in pastures for both years (Fig. 3a). When we give each species a proportional weight relative to its abundance (q1), we did not observe significant differences in the effective number of species between forests from 1977, 2018 and 2019. However, these forests have a greater bee diversity than that observed in the pastures of both 2018 and 2019, which do not show differences between them (Fig. 3b). When we consider only the abundant species, (q2), we observe that the forests of 1977 have a lower effective number of species and less evenness than the forests of 2018 and 2019, but greater than the pastures of those same years (Fig. 3c).

Four species reported in forest fragments from 1977 did not appear in those same forests in 2018 and 2019, all belonging to the genus *Euglossa* (*Eg. azureoviridis*, *Eg. bursigera*, *Eg. hansoni*, *Eg. hemichlora*). However, in 2018 and 2019 we found three species not reported in 1977; an individual from *Eg. allosticta* in both years, one of *Eg. ignita* in 2018 and one of *Eg. sapphirina* in 2019. In the present and previous study, *Eg. dilemma* was the most abundant species (Fig. 4). The next most abundant species for 1977 was *Eg. hemichlora* (not currently reported) and *Eg. variabilis*, which together with *Eg. dilemma* represents 80% of all individuals collected at that time (Fig. 4). For 2018 and 2019, *Eg. dilemma*, *Eg. imperialis*, and *Eg. tridentata* are the most abundant species, contributing to the total species by 82% for 2018 and 84% for 2019. At pasture-dominated sites, we again find that *Eg. dilemma* is the most abundant species followed by *Eg. tridentata* and *Eg. variabilis* in 2018, reversing the order of these last two in 2019, and which represent approximately 90% of all individuals for both years (Fig. 4).

The ADONIS analysis of bee species frequencies shows that habitat type and year affect euglossine bee community composition (F= 3.86, R^2=0.39, df= 4, p=0.001). The forest community of 1977 is more similar to the pastures of 2018 and 2019 (Fig. 5). The result is associated with similar abundances of the common species in both sites as *Eg.*

dilemma, *Eg. tridentata*, *Eg. variabilis* y *Eg. imperialis*, *Eg. townsendi*, *El. cingulata* y *El. meriana* (Fig. 4). In the same analysis, we observe that the community composition changes during the year (F= 4.09, R^2=0.30, df=3, p= 0.001, Fig. 5). *Eg. dilemma* was the most abundant species in all seasons, while the abundance of the other species varied throughout the year. For example, the second most abundant species in the early and mid-rainy season was *Eg. tridentata*, while at the end of the rainy season and the beginning of the dry season it was *Eg. imperialis*.

According to the analysis of indicator species, *Eg. imperialis* (A = 0.97, B = 1, IndVal = 0.98, p = 0.001), *Ex. smaragdina* (A = 0.94, B = 0.75, IndVal = 0.84, p = 0.003) and *El. meriana* (A = 0.91, B = 0.58, IndVal = 0.73, p = 0.029), could be used as indicator species of forest habitats in the current TDF. For example, when we break down the components of *Eg. imperialis* indicator value, we can see that if we find *Eg. imperialis* in a site, the probability that it's a forest is 97% (A), while if we are in a site previously classified as a forest, the probability of finding *Eg. imperialis* is 100% (B). The rest of the species do not seem to be specifically associated with any type of cover.

We found variation of euglossine diversity throughout the year. For all the years (1977, 2018, and 2019), there was a slight decrease in richness and abundance of individuals in the middle of the dry season (March). Additionally a slight increase in general abundance in the middle of the rainy season (August), while 40 years ago it occurred at the end of that same period (December) (Appendix: Table A2). We observed six dominant euglossine bees species in TDF during 2018 and 2019: *Eg. dilemma*, *Eg. imperialis*, *Eg. tridentata*, *Eg variabilis*, *El. meriana* y *Ex. smaragdina*. All these species had fewer individuals in the pastures (Fig. 6, Table 2). *Eg. imperialis* shows the greatest decrease in pasture abundance, while in *Eg. dilemma* the negative effect is weaker (Fig. 6, Table 2). Regarding seasonality, the peak of abundance for *Ex. smaragdina* was March, while *Eg. dilemma*, *Eg. tridentata*, and *Eg. variabilis* were more abundant in August, *El. meriana* showed greater abundance in June, and *Eg. imperialis* abundance increased in December (Fig. 6, Table 2).

DISCUSSION

We found that between 1977 and 2018-2019 period, the number of species decreased slightly (three fewer species for 2018 and four for 2019), which represents a 15% reduction in the number of species seen 40 years ago. However, new rare species were detected in 2018-2019. Rarefaction analysis of species diversity shows that the difference between sampling periods is not significant. The slight decrease or the similar diversity of bee species is not what we expected that the increase in forest cover in the region since 1977 would increase euglossine diversity. Three long-term studies carried out both in protected forest areas and urban areas, it has been determined that the orchid bees populations show long term stability, compared to other groups of insects (Roubik and Ackerman, 1987; Roubik, 2001; Nemésio *et al*. 2015). However, Vega-Hidalgo *et al*. 2020) demonstrated that even in areas without evident human pressures, this group of bees shows a strong decline in its populations. It is possible that the stability in richness and abundance of euglossine bees observed by studies in tropical regions has been a result of a balance between positive (increasing forest cover) and negative factors (land-use change, global warming, invasive bee species, pesticide use) affecting euglossine bee populations (Roubik *et al*., 1986; Moritz *et al*., 2005; Paini, 2004; Chapagain, 2011)

Despite of the tendencies mentioned above, the present study shows a replacement of species in the TDF of Costa Rica. Four species from 1977 did not appear in the recent census, but we found three species not reported in 1977. For instance, *Eg. hemichlora* was the second most abundant in 1977, but it was absent in current censuses. This species occurs from Mexico to Colombia (Roubick and Hanson, 2004). But is rarely reported (Ackerman 1989, Ramírez *et al*. 2002) and consequently there is little information about its response to habitat loss. Some studies have argued that species at the edge of their climatic distribution are more prone to decline (Williams, 2005; Arbetman *et al*., 2017). This could apply to the absence of the other three species in the current census (*Eg. bursigera*, *Eg. hansoni* and *Eg. azureoviridis*), for which Costa Rica is the southern limit of their distribution (Roubik and Hanson 2004).

We found that land-use change (forest by pastures) affects euglossine diversity. All abundant species had fewer individuals in pastures. With fewer floral resources, higher temperature, and lower humidity in open places such as pastures, these habitats could pose

a barrier to foraging for many bee species (Morato, 1994; Tonhasca *et al*., 2002; Cândido *et al.*, 2018). We found three species closely associated with forests—*Eg. imperialis*, *El. meriana*, and *Ex. smaragdina*—and therefore propose that they are good indicators of these sites. Previously, *Eg. imperialis* has been proposed as a bioindicator of conserved sites due to its highly forest-dependent forraging range (Mateus *et al*., 2015; Rosa *et al*., 2015). Likewise, the increase in abundance of *Eg. imperialis* in the current censuses could be associated with the increased forest cover in ACG during the last decades (Calvo *et al*., 2009). In the Atlantic Forest of Brazil changes in the opposite direction have been described, where the euglossines bees associated with forests have declined, while species associated with altered habitats have increased in abundance (Nemésio, 2013).

As we originally hypothesized, the euglossine community in pastures in SRNP is more similar to the 1977 community than that of current forests. Is is possibly related to the similar abundance of species such as *Eg. townsendi, Eg. dilemma*, and *Eg. tridentata*. In the case of *Eg. towsendi*, the pastures do not affect its abundance. In particular, this species has been more associated with small fragments of forest, in contrast to other species (Cándido *et al*., 2018). For *Eg. dilemma*, the pastures show a slight negative effect. This species is well studied and stands out for preferring hot, dry environments and is resistant to highly degraded sites (Eltz *et al*. 2011). Another species tolerating the pastures in this study was *Eg. tridentata*, a bee described as highly generalist in terms of fragrances used, which may favor the colonization of altered sites (Ackerman, 1989; Rosa *et al*., 2015).

It has been proposed that larger bee species, with high flight capacity, should be less affected by fragmentation and habitat loss (Greenleaf *et al*., 2007). In this study, the three most abundant euglossine bee species in pastures were small, while the three forest indicator species were the largest species found. Similar results were observed previously in other studies, where the smallest bees were found in disturbed areas dominated by crops, while the largest bees were never found outside the forest (Milet-Pinheiro and Schlindwein, 2005; Rosa *et al*., 2015). The smaller bee species can tolerated to the harsh conditions outside forests because they need fewer resources for their offspring (Cane *et al*. 2006). Some euglossine bee species may also have more sensitive thresholds to foraging in open pastures. We found that large euglossine bee species avoid fragance traps located at the forest border of SRNP (grassland in Rosa María Ranch). This clearly shows that some

euglossine bee species avoid open areas, even when there is an attractive resource available within their foraging range. Territory fidelity by male euglossine bees is another behavioral pattern that can explain species composition change over short distances in response to vegetation changes (Armbruster, 1993; Pokorny *et al*. 2016, Coswosk *et al*., 2017).

We observed a seasonal change in the peak abundance of some euglossine bee species in TDF, as also noted 40 years ago (Janzen *et al*. 1982). In 2018 and 2019, most species increased their abundance during the middle of the rainy season (August), while in 1977, the peak occurred at the end of the rainy season (December). Other studies observed similar patterns, where the greatest diversity and abundance of euglossine male bees occurs in the rainy season, a pattern associated with the variation in the amount and type of floral resources throughout the year (Dressler 1982; Frankie *et al*. 1983; Andrade-Silva *et al*., 2012; Ramírez *et al*., 2015). Janzen *et al*. (1982), proposed that the seasonal fluctuation in euglossine diversity is due to the individuals´ movement from lowland dry forest to highland humid forests, pursuing resources available in wet habitats. Others have proposed that abundance changes are instead due to the synchronous emergence of bees, and not to mass migration (Ackerman, 1983; Roubik and Ackerman, 1987).

Seasonal fluctuations of euglossine bee populations can be affected also by climate change, since it affects aspects of bee phenology such as the length of the larval development, voltinism, and diapause of some bee species (Forrest, 2016). Furthermore, this would affect the synchronization between pollinators and their plants, which becomes more serious in a relationship as specific as the males of euglossine bees and their orchids. We currently observed a small temporal change in the population peak among euglossine bees within SRNP compare with censuses of 1977. If a stable tendency exists, that change could be related to various factors such as climate change, community variation, or land changes during the recent years.

We observed the loss of three or four euglossine bee species in the TDF of Costa Rica throughout these 40 years, which could be considered to be a slight reduction. Detecting few rare species in the current census probably avoids that species richness is not significantly different than found 40 years ago. However, there are apparent changes in community structure, such as species replacement (including one of the most abundant species in 1977), greater evenness, and the rise of forest-depend species. Those changes in

the community structure and the similarity between the 1977 bee forest community and the pastures samples in 2018-2019 agree with our initial hypothesis about the possible effect of forest recovery on the bee community. We can conclude that the expansion of secondary forests in the SRNP since 1977 has favored forest-associated euglossine bee species. It is difficult to say if this tendency could lead to the recovery of the original bee community of the intact TDF before deforestation, especially under the new circumstances resulting from climate change.

Even though none euglossine bee species reported here is endemic to TDF, the euglossine bee community structure and composition is unique. Euglossine bee plays a vital role in TDF, especially by providing pollination to many plant species with large-bee flower pollination syndrome (Frankie et al., 1983). The highly seasonal TDF of Central America, which today conserves only 1.7% of its original surface and which is strongly threatened by climate change (Calvo-Alvarado *et al*., 2009; Griscom & Aston, 2010; Janzen & Hallwachs, 2019), therefore monitoring changes in the bee community is particularly crucial to document the effects of conservation efforts to increase the TDF protected area. The present study shows that shows that the conservation initiatives promoted in the ACG, such as the increase in the protected area has rendered valuable conservation effects in a insect group that otherwise could have been more heavily degraded. Long-term monitoring of bee populations would be invaluable in elucidating the community dynamics and help to propose more accurate management and conservation plans.

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TABLES

Table 3. Generalized linear models of the effect of habitat and sampling month on bees for six dominant species of euglossine bees from the dry tropical forest of Costa Rica in 2018 and 2019. Coefficient, intercept, Z value, and the probability of the test are shown. The intercept represents the basal level in March in the forest habitat.

Species		Month			Habitat
	Intercept	June	August	December	Pastures
Eg. dilemma					
Estimated	2.89	0.35	1.53	0.71	-0.43
Z	21.24	2.00	10.21	4.28	-2.00
P	**<0.001**	**0.046**	**<0.001**	**<0.001**	**0.046**
Eg. imperialis					
Estimated	2.38	-0.09	0.09	1.13	-3.48
Z	13.58	-0.38	0.36	5.63	-8.40
P	**<0.001**	0.706	0.718	**<0.001**	**<0.001**
Eg. tridentata					
Estimated	1.77	0.67	0.83	0.09	-1.67
Z	8.01	2.49	3.16	0.30	-7.04
P	**<0.001**	**0.013**	**0.001**	0.763	**<0.001**
Eg. variabilis					
Estimated	0.97	0.65	1.16	0.74	-0.95
Z	3.16	1.74	3.35	2.01	-4.06
P	**0.001**	0.082	**0.001**	**0.044**	**<0.001**
El. meriana					
Estimated	-0.50	2.08	-0.69	0.69	-2.35
Z	-0.70	2.77	-0.57	0.80	-3.18
P	0.48	**0.005**	0.571	0.423	**0.001**
Ex. smaragdina					
Estimated	1.84	-0.92	-1.20	-2.30	-2.83
Z	8.10	-2.19	-2.59	-3.10	-3.89
P	**<0.001**	**0.028**	**0.010**	**0.002**	**<0.001**

FIGURES
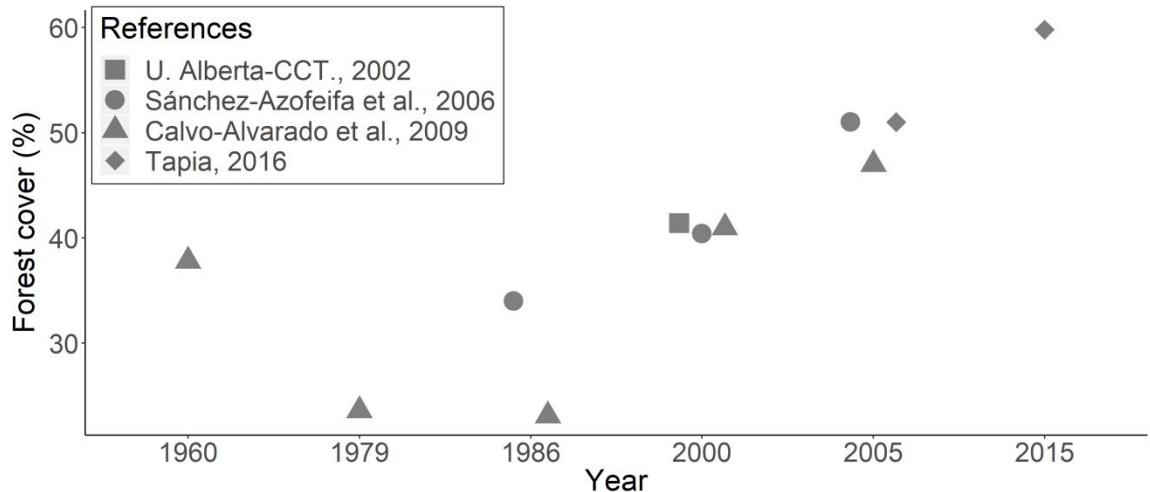
Figure 12. Forest cover estimated in Guanacaste province, Costa Rica, from 1960 to 2015.
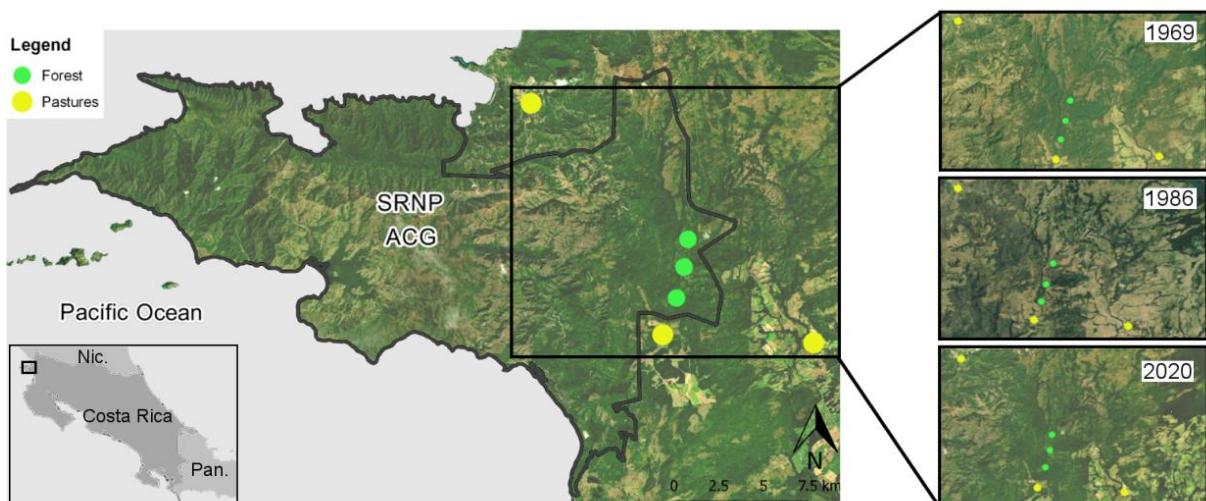
Figure 13. Map showing forest sites (green circles) and pasture-dominated sites (yellow circles) used in this study and located in the Santa Rosa National Park (PNSR) within the Área de Conservación Guanacaste (ACG). The three images to the right of the map are satellite images of SRNP in three different years (1969, 1986, 2020), where greenish areas are forest, yellow-green areas are pastures and circles are sampling sites. A strong reduction in forest covering can be observed in 1986.
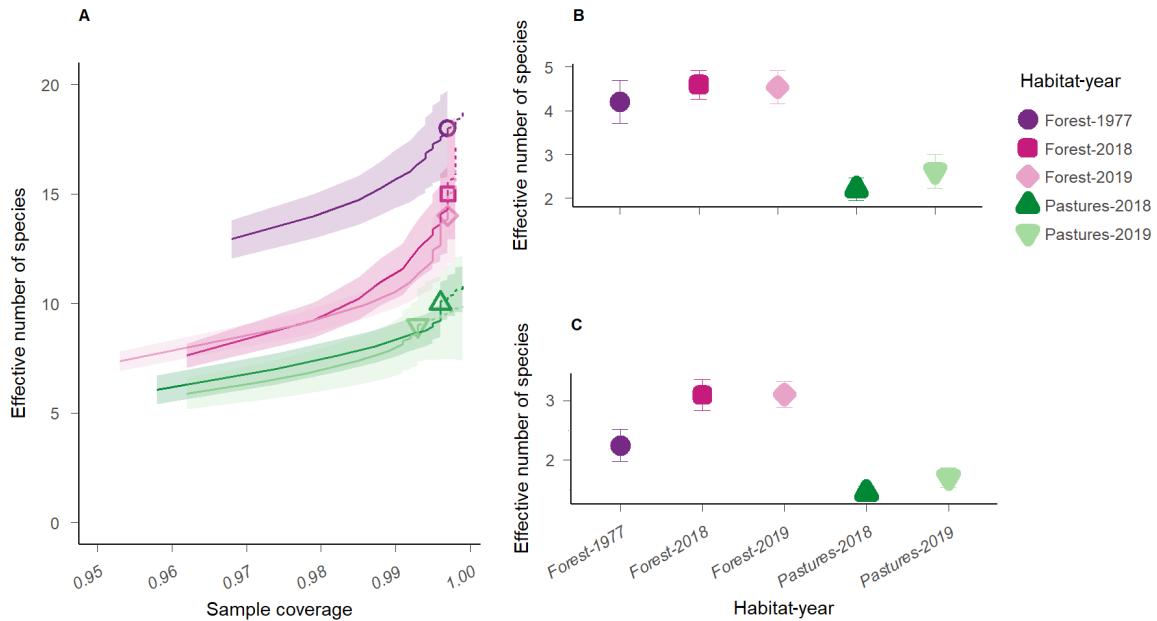
Figure 14. The effective number of euglossine bee species from dry tropical forest in 1977 forests and 2018 and 2019 forests and pastures using Hill's numbers. A: species richness (q = 0) in the sampling coverage range 95-99 %. The shaded area (A) represents the calculated standard deviation and dotted lines indicate the extrapolation of the number of species for 1500 individuals. B: effective number of typical species (q = 1) and C: effective number of dominant species (q = 2) at 99% sampling coverage and the lower and upper limit of standard deviation.
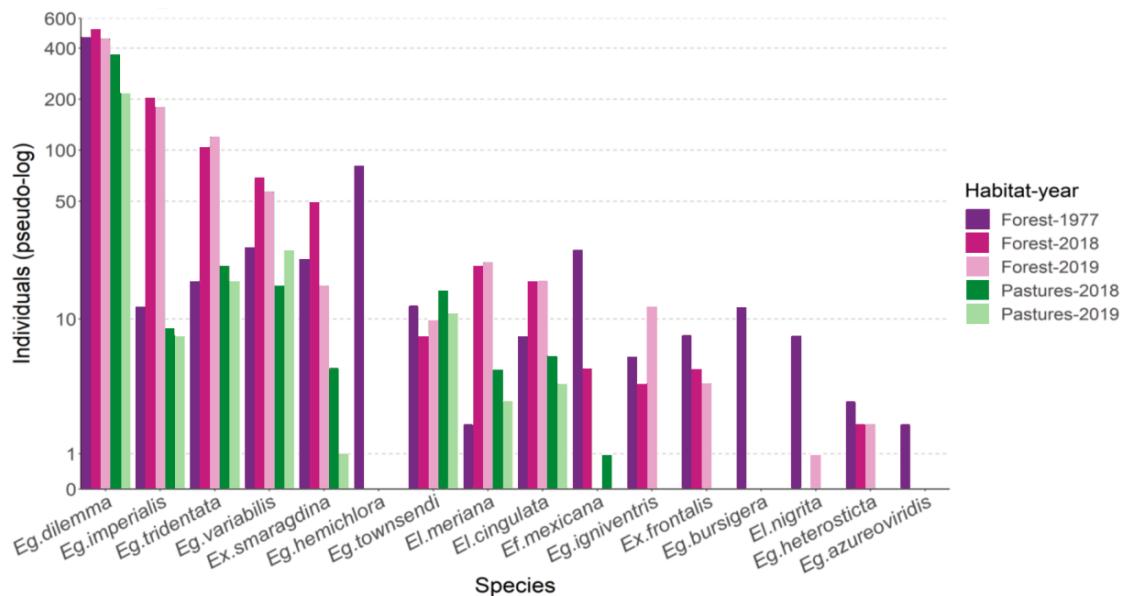
Figure 15. Individuals for each species of euglossine bees found in the forests of 1977 and forests and pastures of 2018 and 2019 in the dry tropical forest of Costa Rica. Species ordered from largest to smallest number of individuals for 2018 and 2019. Note that the y axis is on a logarithmic scale. Only species with an abundance > 1 in the year are represented.
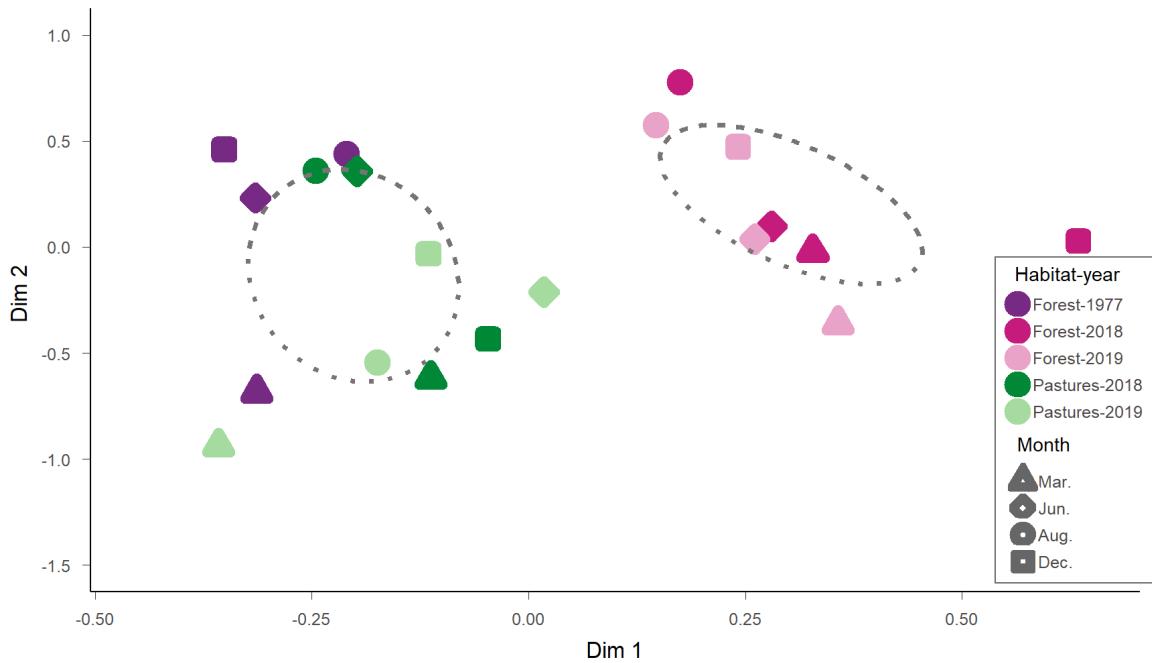
Figure 16. Non-metric multidimensional scaling (nMDS) using Bray-Curtis distance for the euglossine bee community composition in 1977, 2018 and 2019 in the dry tropical forests of Costa Rica (k=3, stress= 4.45).
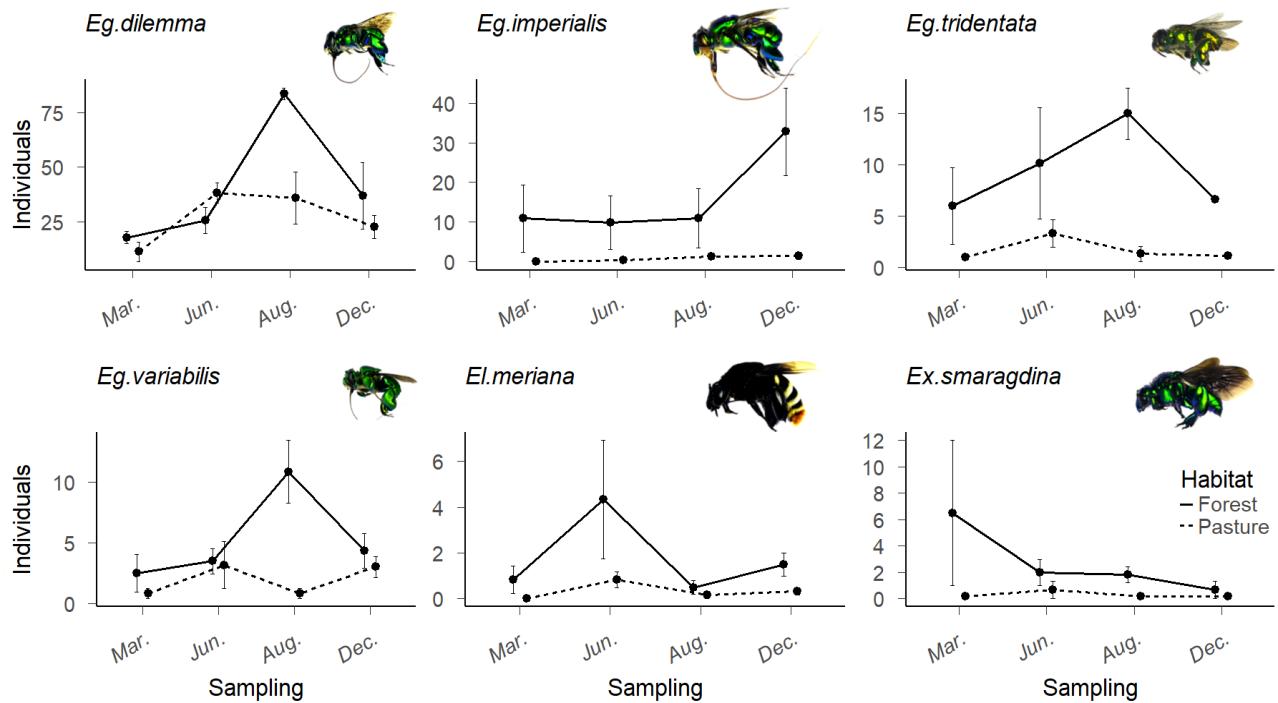
Figure 17. Average abundance (± standard error) for the six abundant euglossine bee species in forests (solid line) and pastures (dotted line) of the tropical dry forest of Costa Rica, during 2018 and 2019.

APPENDIX

Table A1. List of species observed in forests and pastures in the Tropical Dry Forest of Costa Rica during the years 2018 y 2019.

	Abundance		
Species name	Forest	Pastures	Total
Eufriesea mexicana (Mocsáry, 1897)	5	1	6
Eufriesea schmidtiana (Friese, 1925)	1	0	2
Eufriesea surinamensis (Linnaeus, 1758)*	1	0	1
Euglossa allosticta Moure, 1969	2	1	3
Euglossa dilemma Bembé y Eltz, 2011	984	634	1618
Euglossa heterosticta Moure, 1968	4	0	4
Euglossa ignita Smith, 1874	1	0	1
Euglossa igniventris Friese, 1925	16	0	16
Euglossa imperialis Cockerell, 1922	387	17	404
Euglossa townsendi Cockerell, 1904	18	27	45
Euglossa tridentata Moure, 1970	227	40	267
Euglossa sapphirina Moure 1968	1	0	1
Euglossa variabilis Friese, 1899	127	47	174
Eulaema cingulata (Fabricius, 1804)	34	10	44
Eulaema meriana (Olivier, 1789)	43	8	51
Eulaema nigrita Lepeletier,1841	1	0	1
Exaerete frontalis (Guérin-Méneville, 1845)	9	0	9
Exaerete smaragdina Guérin-Méneville, 1845	66	7	73
Total	1926	793	2719

* This species was observed in this study but was not taken into account in the statistical analysis.

Table A2. Richness and abundance of the euglossine bee community by habitat-year and month in a dry tropical forest of Costa Rica.

Habitat-year	Measure	Mar.	Jun.	Aug.	Dec.
Forest-1977	Richness	7	11	12	10
	Abundance	60	174	217	269
Forest-2018	Richness	7	10	10	8
	Abundance	165	183	452	219
Forest-2019	Richness	7	11	12	8
	Abundance	106	170	329	302
Pastures-2018	Richness	5	8	9	9
	Abundance	49	193	189	71
Pastures-2019	Richness	3	8	6	6
	Abundance	29	95	55	112

CAPÍTULO 2

Formato para la revista: -

Body size variation of *Euglossa dilemma* **Bembé and Eltz (Hymenoptera: Apidae: Euglossini) related to habitat loss**

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Abstract

Body size is a highly plastic functional trait that responds to both genetic and environmental factors. For bees, this trait has been found related to the individual's fitness. When resources are limited, it has been proposed that females may choose to reduce or not the offspring size, this in order to maintain fertility or maintain the fitness of each individual, respectively. In areas such as pastures, environmental conditions and the scarcity of available resources, becomes a barrier for many bee species. However, bees like *Euglossa dilemma*, a species of the Euglossini tribe, are able to use this type of habitats. We ask ourselves the following questions Are differences in the mean and magnitude of variation in *E. dilemma* body size in forest and pasture habitats? And, how does the season affect this trait? We use *E. dilemma* individuals captured in forest and pastures of tropical dry forest in Santa Rosa National Park, Costa Rica. We use six measurements of body size. Using glmm, we did not find differences in the mean of any of the measurements of the *E. dilemma* body size, between individuals collected in forests and pastures. This shows that mean body size in one direction or another in response to habitat loss is not the rule for all bee species. Based on most of the body measurements of *E. dilemma*, we observed the largest individuals at early-rainy season, while the smallest at late-rainy season. However, we observe differences in body size and population size in forests throughout the year; this is possibly associated with fluctuation in flower resources. Likewise, we observe that the variation in size decreases in degraded sites. The loss of variation in body size could be a sign of negative effects that habitat loss is having on *E. dilemma* populations.

Key words: Functional traits, habitat loss, land-use change, euglossines, resilience.

INTRODUCTION

Insect body size is a highly plastic trait and one of the most studied modifications, since it is affected by environmental and genetic factors (Honěk, 1993, Davidowitz *et al*., 2003). Environmental temperature and the quantity and/or quality diet in larval development are two proximate causes that explain the species body size variation (Bosch and Vicens 2002; Davidowitz *et al*., 2003). Also, this trait is indirectly related to fitness and replaces the measurement of other traits whose measurement is harder (Violle *et al*., 2007). In bees, this trait is associated with characteristics such as: flight range (Greenleaf *et al*., 2007), foraging periods (Wilmer and Finlayson, 2014), immune response (Arriaga *et al*. 2017), fertility (Honěk, 1993), diet specialization (Bommarco *et al*., 2010), among others.

For most tropical bees, large homogeneous areas of grasslands or crops become a barrier, as they generally have high temperatures, low humidity, and less available resources (Bennett and Sauders, 2010). It has been proposed that given the scarcity of flower resources, females could maintain fertility at the cost of reducing cell provisioning, thereby reducing the offspring size (Rosenheim *et al*., 1996). In this way, habitat loss could modify the mean and/or body size variation of bee populations in degraded sites. For the mean size, a consistent pattern is not reported (Benjamin *et al*., 2014; Williams *et al*., 2010; Forrest *et al*., 2015; Warzecha *et al*., 2016; Ribeiro *et al*., 2019), while for the variation magnitude, it can be expected to decreases when conditions are unfavorable, due to decrease in genetic and phenotypic variability (Lande and Barrowdough, 1987; Weiher and Keddy, 1995). However, when resources are limited, females may also choose to reduce their fecundity but maintaining their offspring size, which would increase the fitness of each individual but decrease the population size in certain sites or seasons (Rosenheim *et al*., 1996; Bosch and Vicens, 2006).

Despite the adverse conditions present in places without forest cover, there are bees capable of using these spaces. It has been pointed out that some bees from the Euglossini tribe have high resilience to natural habitat fragmentation and are capable of using forests and open areas, but a clear pattern has not been demonstrated (Nemesio, 2009; Moreira *et al*. 2017). An example is *Euglossa dilemma*, a sister species of *Euglossa viridissima*, whose differences are mainly based on teeth number in the jaw and the chemical components they

collect (Eltz *et al*., 2011). For both species, Mexico is the northern limit of their distribution. However, the southern limit of *E. viridissima* is Honduras, while for *E. dilemma* is Costa Rica (Eltz *et al*., 2011). In terms of habitat preferences, it is known that both species prefer dry and hot areas and can tolerate sites with a high degree of disturbance (Eltz *et al*., 2008, Zimmermann *et al*., 2011), making them a good study model.

Based on this, we ask ourselves the following questions i) Are differences in the mean and magnitude of variation in *E. dilemma* body size in forest and pasture habitats? and ii) How does the season of the year influence body size and abundance of *E. dilemma* in forests and pastures? Related to thermoregulatory ability and availability of resources, we would expect smaller individuals in pastures, due to small bees lose heat more easily, and so they can avoid overheating, at the same time require fewer resources for their diet (Peat *et al*., 2005a; Nooten and Rehan, 2020). Likewise, the environmental conditions in pastures would lead to greater abiotic stress and smaller populations, resulting in a decrease in the magnitude of the body size variation of *E. dilemma* (Hulshof *et al*., 2013). Also, due to the variation in the floral resources available throughout the year, we expect that the *E. dilemma* females will change the provision investment, and therefore, we will observe differences in the body size and/or abundance of this species (Bosch, 2008).

MATERIALS AND METHODS

Study site- We carried out this study during the years 2018 and 2019 in the Santa Rosa Sector (known as SRNP in1977) in the Área de Conservación Guanacaste (ACG) and adjacent pastures, in the northwest of the Guanacaste province, Costa Rica (10° 45' -11° 00' N, 85° 30' - 85° 45' W, Fig. 2), on the SRNP plateau at 300 m altitude. SRNP comprises two types of forest: the evergreen forest dominated by oak trees (*Quercus oleoides*) and tropical dry forest (TDF) (Hartshorn, 1983). The TDF of SRNP has an average annual temperature range of 26.6 to 27.5 ° C, and receives an average rainfall of 1390-1800 mm annually, with a high degree of year-to-year variation (Gillespie *et al*., 2000; Janzen, 2000; Magnani, 2018). The TDF is characterized by a marked seasonality, with 6 dry months ranging from late December to mid-June, a period in which most woody plants lose their leaves (Janzen, 1993; Gillespie *et al*., 2000).

*Sampling sites-*We selected the three sampling sites that corresponded to fragments of TDF located at 300 m altitude in SRNP. The first site is a deciduous regenerating secondary forest, the second is a semi-evergreen forest and the third is an oak forest (Janzen *et al.* 1982). For grassland censuses, we selected three open grass-dominated sites. The first site is located 5.5 km from the limits of SRNP, near the Inter-American highway intersection with Quebrada Grande, the second site is in Las Melinas neighborhood in Cuajiniquil 2.8 km from SRNP, and the third one at Hacienda Rosa María 1.5 km south of the Casona de Santa Rosa, approximately 500 m away from the protected area (Fig. 1). These pastures were at approximately the same altitude as the forest sites and we used the same collection periods and methods.

Experimental design- We sampled from 7 to 11 a.m. in the mid-dry season (March), beginning, middle, and end of the rainy season (June, August, and December, respectively). We also used the same five chemical attractants: cineol, eugenol, methyl cinnamate (solid), benzyl acetate, and methyl salicylate, 99% pure from Sigma-Aldrich brand. We moistened cotton balls of approximately 5 cm diameter with 3 ml of each attractant and placed them 1.5 m above the ground hanging from branches of trees or shrubs. In the case of pastures, we used a living fence to hang cotton. We performed the following body size measurements: Head width (HW), interocular distance (ID), mesosoma length (ML), intertegular distance (ITS), wing length (WL), wing width (WW), and glossa length (GL) (Fig. 2). For this, we used a Leica brand stereoscope, model (¿?) with an integrated camera, for measured digitally each distance.

Statistical analysis: For tests and comparative graphs, we tabulated the measurements of *E. dilemma* body size by sampling period and habitat (forests or pastures). We used the R program (R Core Team, 2019) for statistical analysis and the specific packages are mentioned in each specific analysis. We compared each body size measurement of *E. dilemma* between forest-caught individuals with the pasture-caught. The body measurements did not meet the assumptions of normality and the transformation could not solve this problem to perform parametric tests. To evaluate season effect on each type of habitat we used generalized mixed models, with a gamma probability distribution and adjusting the models with the method *penalized quasilikelihood* (PQL) for parameters estimation, due to the lack of normality in data and residuals. We use the habitat type and

sampling period (month) as fixed variables. Because each sampling site had its own environmental and spatial conditions, we used the sampling site as a random variable. For this analysis, we use the glmmPQL function from the package *MASS* (Venables and Replay, 2002). We use Fisher's exact test of independence to find out if the abundance of *E. dilemma* in each sampling period differs between forests and pastures. Finally, to compare the variance of each measurement between habitat types we use Lavene test.

RESULTS

We registered 937 individuals of E. dilemma, 523 in forests, and 414 in sites dominated by pastures, of these we collected 109 specimens for measurements, 61 individuals for forests and 48 for pastures. The interocular distance and the mesosoma length showed a high correlation (r ~ 0.8) with the head width and the intertegular distance, respectively (Appendix: Fig. 1a), for which we only considered six measurements in the analysis: head width (HW), distance intertegular (ITS), glossa length (GL), width (WW) and wing length (WL) and relative wing length (RWL)

We did not observe differences in the mean length of any of the body size measurements between individuals of *E. dilemma* captured in forests and pastures (Fig. 3, Table 2). When we consider the sampling period and the habitat type we find that the sampling period affects the mean length of the body size measurements of *E. dilemma* (Fig. 3, Table 2). Four measurements (HW, WL, WW and GL) presented greater length in June (early-rainy season), compared to the month of March (mid-dry season), while the ITS and GL presented a shorter length in December (late-rainy season) (Fig. 3, Table 2). Only in the ITS, the habitat type dominated by grasses have a positive effect for December, the other traits didn't show a relationship with the sampling month and the habitat (Fig. 3, Table 2).

We observed that the abundance of *E. dilemma* in each sampling month is dependent on the habitat type (Fisher's Test: p <0.001), with a greater abundance in forest habitat (Fig. 4). In the forest, we observed the least amount of individuals of *E. dilemma* in the mid-dry season (March), while in the mid-rainy season (August) there was an increase in abundance. The abundance in grasslands remains more or less constant (Fig. 4).

Regarding the variation of the body size measurements of *E. dilemma* in different habitats, we found that all measurements (ITS: F= 7.06, n= 92, p= 0.009; HW: F= 4.27, n=

92, p= 0.042; WL: F= 8.40, n= 92, p= 0.004; GL: F= 6.86, n= 92, p= 0.01; RWL: F= 6.28, n= 92, p= 0.014), except wing width (WW: F= 1.63, n= 92, p= 0.205), had a higher variance in individuals captured in forests compared to individuals from sites dominated by pastures (Fig. 5).

DISCUSSION

Contrary to expectations, we did not find differences in the mean of any of the measurements of the *E. dilemma* body size, between individuals collected in forests and pastures. Previous research on the relationship between bee body size and habitat loss does not show a consistent pattern. Brito *et al*. (2018) conducted a comparison of the orchid bees body size between forests and large palm plantations in the Brazilian Amazon, finding that males were ~14% bigger in plantations. In this and other studies, they argue that this is because bigger bees have greater flight capacity and transfer heat more efficiently, allowing them to use deforested areas (May and Casey, 1983; Pereboom and Biesmeijer, 2003; Greenleaf *et al*., 2007; Brito *et al*. 2018). Other bees have shown the opposite pattern, as land use increased, mean body size decreased (Geslin *et al*., 2016; Renauld *et al*., 2016). Among other explanations, these authors associate these results with habitat isolation and the decrease of quantity and quality resources, which affects the efficiency in the female foraging and therefore, the size of its progeny (Forrest *et al*., 2015; Renauld *et al*., 2016).

Even species from the same Euglossini tribe differ in the phenotypic plasticity shown by the land-use change (Silva *et al*., 2009; Ribeiro *et al*., 2019). Ribeiro *et al*. (2019) reported that *Euglossa ignita* individuals were larger in smaller fragments and areas with agroforestry crops, while *Euglossa cordata* and *Eulaema atleticana* did not show any pattern. Another study carried out with *Euglossa pleiosticta* and *Eulaema nigrita*, did not show differences in the mean size of either of the two species in response to the degraded area by human activities, but both showed differences in the wing variation asymmetry, *E. pleiosticta* in greater magnitude (Silva *et al*., 2009). This shows that size variation in one direction or another in response to habitat loss is not the rule for all bee species. Having larger bodies favors dispersion and thermoregulation, however, these advantages may not offset the nutritional demand that this represents (Benjamin *et al*., 2014; Classen *et al*., 2017). Whatever the proposals presented to explain differences in mean size between

forests and pasture individuals, we did not observe differences for *E. dilemma*. This shows that the response to environmental stress is highly variable (Atkinson, 1996).

Based on most of the body measurements of *E. dilemma*, we observed the largest individuals at early-rainy season (June), while the smallest at late-rainy season (December). In this site, most of the trees, lianas, and other plants that have adaptations to be pollinated by large bees, such as euglossine, bloom in dry season to early-rainy season (Frankie *et al*., 1983). Furthermore, it has been estimated that in *Euglossa viridissima* (sister species) the ontogeny of males requires on average 63 days (May-Itzá *et al*., 2014). Therefore, the massive flowering in the dry season could favor the cells provisioning, favoring the observation of larger individuals two months later in June. The opposite response has been observed when floral resources are limited since bees tend to decrease the provisioning (Ramalho *et al*., 1998; Geslin *et al*., 2016).

The bee body size is a highly plastic trait that responds largely to environmental factors and a lesser degree to genetic factors (Tepedino *et al*., 1984). The amount of food ingested during the larval phase is the factor that dominates the attributes observed in body size (Bosch y Vicens, 2002; Radmacher y Strohm, 2010). However, temperature plays an important role, since it has been observed that at high temperatures the larvae are smaller. Although the causes of this phenomenon are not clear, it is proposed that the increase in temperature favors the faster develop larvae, so that the hatchlings ingest less food, which leads to a smaller size (Karl y Fischer, 2008; Radmacher y Strohm, 2010). This could be related to the small individuals of *E. dilemma* observed in the early and mid-dry season. Other factors that could also affect the bee body size are competition with other bees such as *Apis mellifera* (Goulson y Sparrow, 2009), parasitism rates (Colla *et al*., 2006), and pesticides use (Bernauer *et al*., 2015).

It is well known that the generations of orchid bees overlap and can live up to 6 months, so the increase in the abundance of *E. dilemma* males may simply be due to the accumulation of generations (Zimmerman *et al*., 2011). At this same study site Frankie et al., 1983 observed a similar pattern, where orchid bees are the most important floral visitors to plants potentially pollinated by large bees, replacing the antphoridae that dominate in the dry season. We observe that immediately after the flowering peak, at the early-rainy season the E. dilemma males are larger, later in the middle of this season we observe a greater

male abundance, while at the end the males are smaller and their quantity dramatically decreases. Similar results were obtained with Dawson's burrowing bees (*Amegilla dawsoni*), since as the flowering season passes, they produce more and smaller males (Tomkins *et al*., 2001; Alcock *et al*., 2005). It is necessary to emphasize that this fluctuation in the E. dilemma abundance is lost in the pastures, possibly associated with the floral resources homogeneity throughout the year (Persson y Smith, 2011).

On the other hand, we observed that the variation magnitude in the *E. dilemma* body size decreased in grasses. Population size is a determining factor in genetic variability and therefore in the phenotypic expression of a population (Lande y Barrowdough, 1987). As we mentioned in the previous paragraph, it is probable that the resources homogeneity throughout the year in the pastures limits the fluctuation in the size population, and therefore also affects the variation in the body size of the individuals. Also, the stress in environmental conditions can results in less variation in traits, due to the genotypic and phenotypic filter (Hulshof *et al*., 2013). The potential environmental stress related to temperature and scarcity of resources may filter the individuals that can be found in pastures and which would ensure a selection towards intermediate sizes, and therefore we do not observe differences in the mean size between both habitats. It has been observed that the intra-specific variation in bees decreases under extreme climatic conditions and scarcity of available floral resources (Torchio y Tepedino, 1980; Classen *et al*. 2017).

E. dilemma, opposite other bee species, did not show any pattern in mean body size in response to habitat loss. However, we observe differences in body size and population size in forests throughout the year; this is possibly associated with fluctuation in flower resources. Likewise, we observe that the variation in size decreases in degraded sites. The loss of variation in body size could be a sign of negative effects that habitat loss is having on *E. dilemma* populations. Furthermore, as has been observed in other studies, the response of *E. dilemma* to habitat loss may be reflected in other aspects such as the form (Silva et al., 2009; Ribeiro et al., 2019), which is why it would be interesting to consider this aspect in future research.

It is necessary to remember that the results are shown here correspond only to males, so it is necessary to consider in the future, the impact that habitat loss is having on E. dilemma females. It has been determined that *E. dilemma* can tolerate highly disturbed

environments. However, we observed that at least the body size variation is affected by changes in landscape use. This shows that even "tolerant" species to habitat loss are affected by fragmentation in the degree of phenotypic variation and possibly adaptability of populations. Body size is one of the first traits to respond to habitat degradation. However, adjustments to this trait, made by individuals of each species, do not prevent population decline (CaraDonna *et al*. 2018, Nooten y Rehan, 2020). Therefore, we consider that it is a key aspect that should be considered in future evaluations on the habitat degradation impact and the insect's global decline.

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TABLES

Table 4. Generalized mixed models using the *penalized quasilikelihood* method with habitat type and sampling month as fixed effects on body size measurements of *Euglossa dilemma* in a dry tropical forest of Costa Rica. The interaction between the variables is only presented in those measurements in which it was significant.

| Trait	Fixed-factors	Value	t-value	p-value
Intertegular span				
Habitat	Pastures	0.003	0.267	0.803
Month	June	0.041	1.875	0.064
	August	0.015	1.285	0.202
	December	-0.045	-3.080	**0.003**
Interactions:	Pastos: June	0.014	-0.480	0.632
	Pastos: August	-0.012	-0.623	0.535
	Pastos: December	0.047	2.057	**0.042**
Head width				
Habitat	Pastures	0.009	1.126	0.323
Month	June	0.044	3.041	**0.003**
	August	0.019	2.135	**0.035**
	December	-0.021	-1.964	0.052
Wing length				
Land cover	Pastures	0.002	0.227	0.832
Month	June	0.050	2.857	**0.005**
	August	0.016	1.499	0.137
	December	-0.027	-2.041	**0.044**
Wing width				
Habitat	Pastures	0.0002	0.02	0.985
Month	June	0.041	2.452	**0.016**
	August	0.036	3.452	**<0.001**
	December	-0.006	-0.447	0.656
Glossa length				
Habitat	Pastures	0.016	1.252	0.278
Month	June	0.055	2.530	**0.013**
	August	0.013	0.880	0.381
	December	0.01	-0.542	0.589
Relative wing length				
Habitat	Pastures	0.004	0.485	0.653
Month	June	0.017	1.150	0.253
	August	0.008	0.933	0.353
	December	0.0003	0.026	0.979

FIGURES
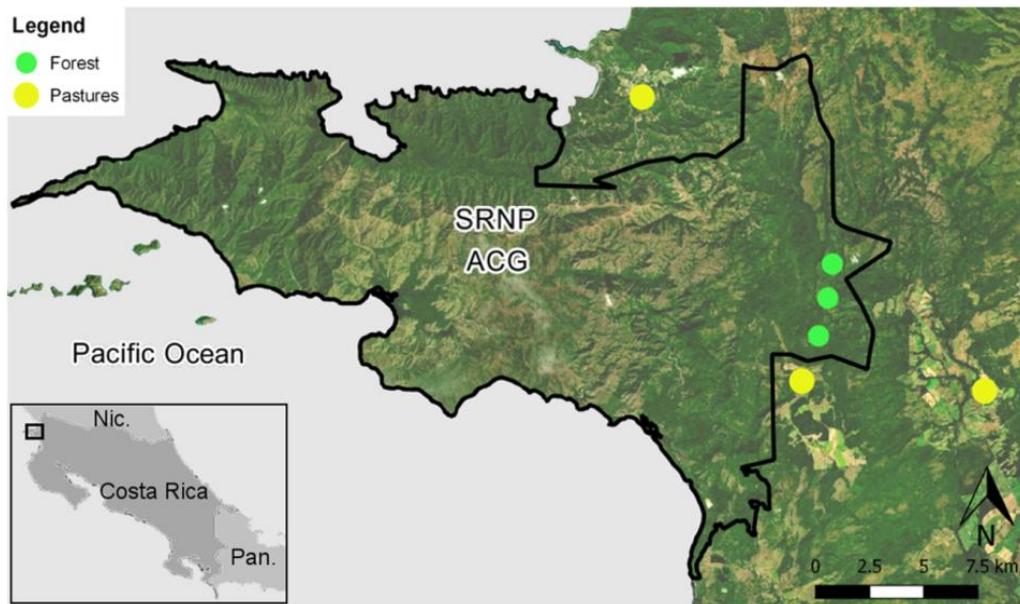
Figure 18. Map showing forest sites (green circles) and pasture-dominated sites (yellow circles) used in this study and located in the Santa Rosa National Park (SRNP) within the Área de Conservación Guanacaste (ACG).
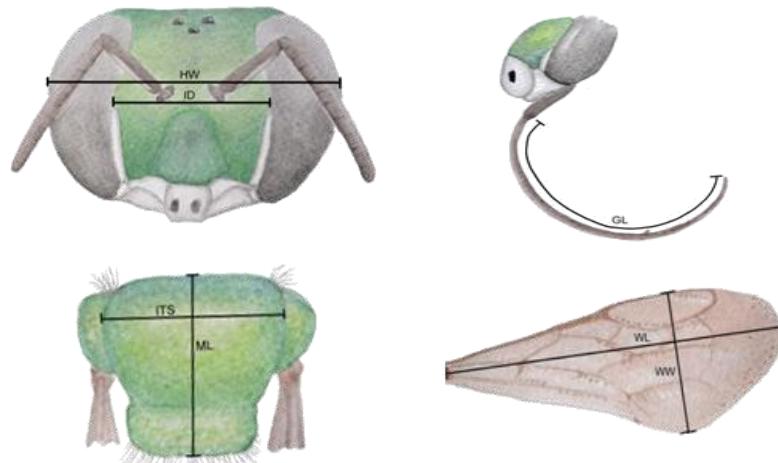

Figure 19. Body size measurements of *Euglossa dilemma*: head width (HW), interocular distance (ID), glossa length (GL), intertegular span (ITS), mesosoma length (ML), wing length (WL), and wing width (WW).
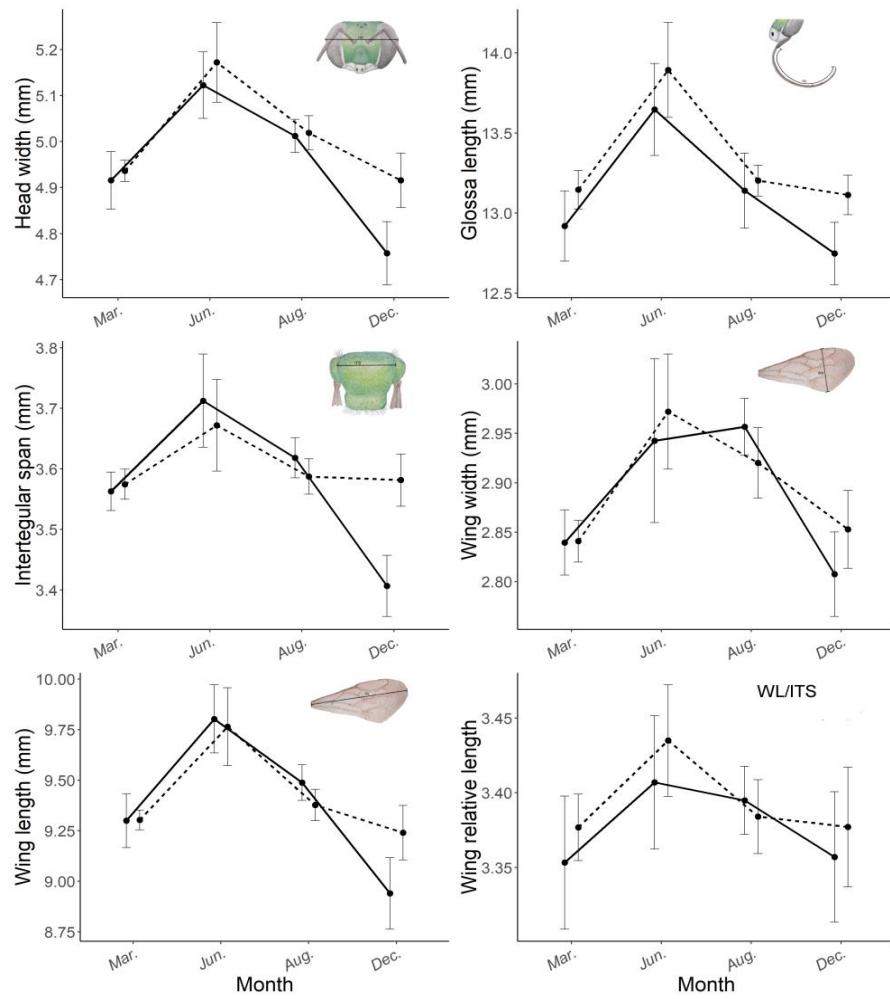
Figure 20. Mean length (± standard error) for body size measurements of *Euglossa dilemma* by sampling month in forests (solid line) and pastures (dotted line) in the dry tropical forest of Costa Rica.
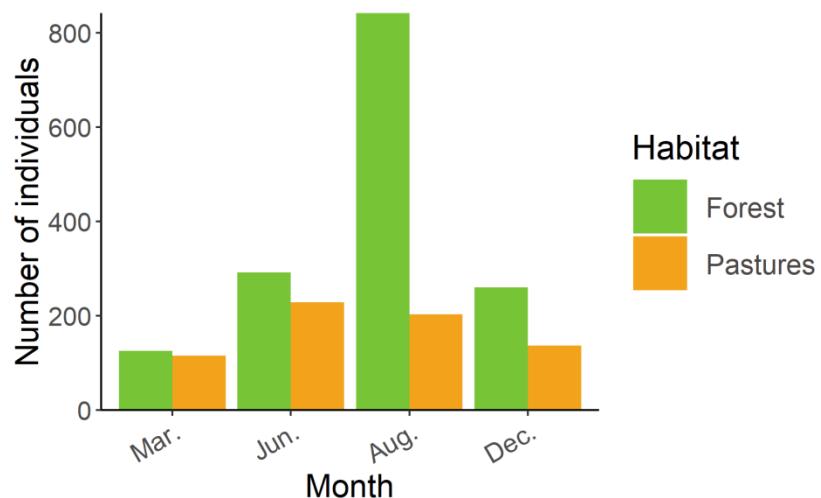
Figure 21. Distribution *Euglossa dilemma* individuals in forests and pastures. Statistics from Fisher's exact test are included showing the distribution comparison.
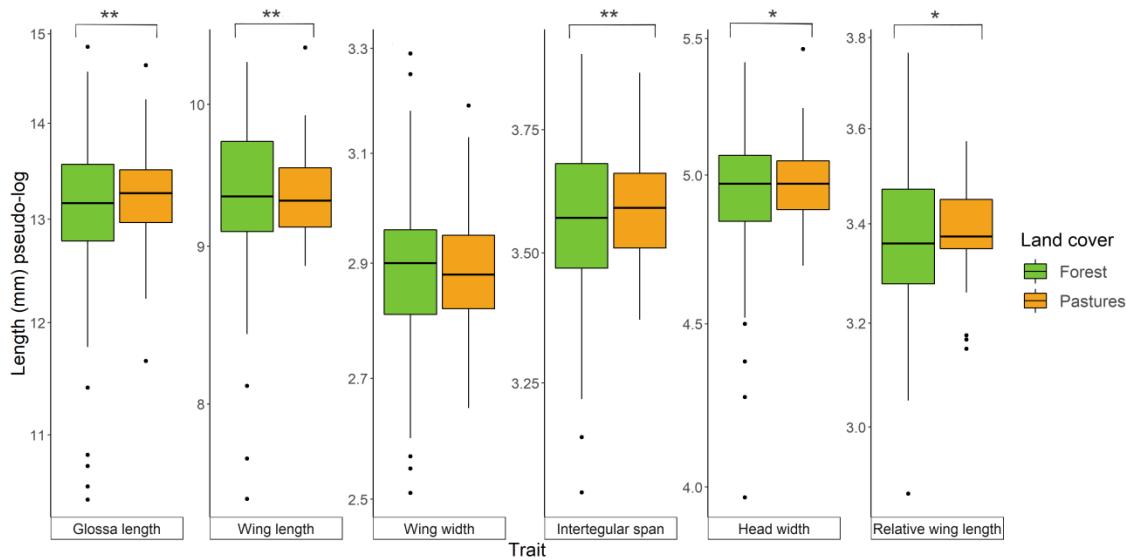
Figure 22. Boxplots showing the distribution of each measurement of *Euglossa dilemma* body size in forests and grasses of the dry tropical forest of Costa Rica. The line in the middle of the box represents the median. The box shows the interquartile range. The * symbols represent the result of the Lavene test for comparison of variances. Significance: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

APPPENDIX

Table 1a. Assumptions test of normality and homogeneity of variances for body size measurements of *Euglossa dilemma*.

Trait	Shapiro- test	Lavene-test
Head width	W = 0.91, **p<0.001**	F=4.5, **p= 0.035**
Intertegular span	W = 0.98, p= 0.184	F=6.19, **p= 0.014**
Wing length	W = 0.96, **p= 0.005**	F=8.51, **p= 0.004**
Wing width	W = 0.97, **p= 0.04**	F=2.76, p= 0.099
Glossa length	W = 0.90, **p<0.001**	F=6.86, **p= 0.010**
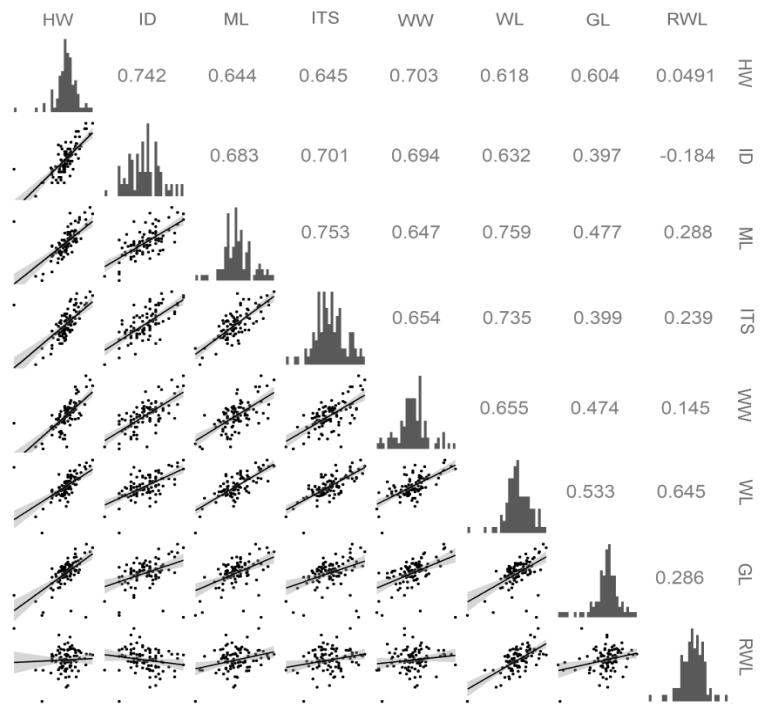
Figure 1a. Correlations obtained between of body size measurements of *Euglossa dilemma.* Head width (HW), Interocular distance (ID), Glossa length (GL), Intertegular span (ITS), Mesosoma length (ML), Wing length (WL) and Wing width (WW).