January 2020

Bee Abundance Along a Tropical Montane Elevational Gradient and Implications for Crop Pollination Services

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Recommended Citation

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BEE ABUDANCE ALONG A TROPICAL MONTANE ELEVATIONAL GRADIENT AND IMPLICATIONS FOR CROP POLLINATION SERVICES

BY

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BY

KRISTIN M. CONRAD

Submitted to the Faculty of the Graduate School of
Eastern Kentucky University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

2020

ACKNOWLEDGEMENTS

I would like to begin by thanking my advisor, Dr. Valerie Peters, whose experience provided the structure for this research and whose guidance motivated my own growth and development. I would also like to thank my committee members, Dr. Jen Koslow and Dr. David Brown, whose contribution of time and expertise was integral to the completion of this thesis. I would like to thank Chelsea Hinton, Jeni Knight, Noelia Solano, Brian Hoven, John Nelson, Clay Thompson, and Taylor Allen, for assistance collecting, processing, and identifying specimens, Tony Robertson for equipment fabrication, and Dr. Sandra Rehan for additional identification assistance. This study was made possible through the financial support from Eastern Kentucky University and the Earthwatch Institute.

The completion of this study would be impossible without the contribution of citizen scientists; I would like to thank all Earthwatch volunteers for their specimen collection efforts and enthusiasm for conservation and adventure. I would also like to express my deepest gratitude to the San Luis, CR community for their hospitality, generosity, and participation that was fundamental to this study, as well as UGA Costa Rica, CIEE, and Maria Hudson for their lodging and accommodation. A special thanks to Alex Jimenez for transportation and logistical assistance.

Lastly, I am incredibly grateful for my family, friends, cohort, and, especially, for Matt Henson and Amber Morris; Your support, assistance, encouragement, and comedic relief helped me to realize my aspirations and I cannot thank you enough.

ABSTRACT

Tropical forests are among the biologically richest ecosystems on Earth, but how most organisms in these forests will respond to a warming climate remains uncertain. Insects are expected to be highly responsive to climate change due to their short life cycles that are strongly influenced by temperature. Plants depend on pollinators to set seed and reproduce, and many animal populations rely on the resources provided by flowering plants. There is an urgent need to document elevational distributions and thermal specialization for tropical bee species to understand how these important pollinators may respond to warming temperatures. My four-year study (2016-2019) aims to quantify bee abundance differences across an elevational gradient. I focus on the two bee tribes comprising the most abundant species collected, Tribes Meliponini and Ceratinini. Bee species of these two tribes are also important pollinators of tropical forest plant species. Three adjacent roadsides, separated by 1 km each, were used as replicate elevational gradients. Each elevational gradient spans 750-1150 m elevation and are located in the seasonally dry Pacific slope forests of Costa Rica. The study area represents a mixture of smallholder farms, pasture, and forest patches, and is located in a conservation area. Bees were sampled at each 100 m elevation change using both active and passive collection methods in an effort to effectively survey the bee community. My results suggest that many species in these tribes exhibit elevational range specialization, and a canonical correspondence analysis indicates that elevation and site explain ~42% of the variation in the community composition of Meliponini and Ceratinini ($F_{pseudo(3,8)} = 1.91$, $p = 0.002$). Mountain-dwelling species are expected to exhibit shifts to higher elevations and cooler temperatures over time due to global temperature increases. My study is one of the first to provide evidence of elevational specialization in several bee species. These findings indicate that bee species of tropical montane landscapes could be at high-risk due to warming temperatures or changing patterns of precipitation, as species with narrow elevational ranges or specialization within a narrow portion of their elevational range are predicted to be more vulnerable to range shifts.

Beyond my assessment of elevation specialization in Neotropical bee species, I conducted an additional study to identify the important pollinators of *Persea americana* and *Cucurbita pepo* within their native ranges. Observations were conducted during December of 2018 and 2019 for *P. americana*, and during the wet and dry seasons of 2019 for *C. pepo*, and resulted in the collection of 145 pollinators visiting *P. americana* and 223 pollinators visiting *C. pepo*. My results suggest that species belonging to the tribe Meliponini are important pollinators for my focal crop species, as they represent approximately 75% of the pollinators visiting both *P. americana* and *C. pepo*; however, species accumulation curves indicate the need for additional sampling in order to accurately determine the entire pollinator assemblage visiting each focal crop species. Community similarity analyses also indicate that elevation may be an important factor in determining the pollinator assemblages of each focal crop species, as a low degree of similarity was detected among communities present at each sampled elevation category. My results support the importance of natural area management, sustainable farming, and conservation of plant- pollinator mutualisms in order to sustain wild bee species abundance and diversity.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SMALL-SCALE PATTERNS OF ELEVATIONAL SPECIALIZATION INDICATE HIGH VULNERABILITY TO CLIMATE CHANGE FOR TROPICAL BEES

INTRODUCTION

Pollination is arguably one of the most essential ecosystem services for humanity globally (Dixon 2009). Specifically, pollination (i) enhances genetic diversity in most plant species, including species that are important for human well-being, such as wild relatives of crops and plant-based pharmaceutical products, (ii) improves the quality and/or quantity of many food crops (Bartomeus et al. 2014, Garibaldi et al. 2013), and (iii) forms the resource base (i.e., fruits, nuts) for wildlife that humans may either derive aesthetic value such as ecotourism, or consumptive value. In lowland forests of the tropics, for example, it is estimated that 98-99% of the plant species producing the food resource base for tropical wildlife rely on animal-pollination (Bawa 1990). Globally, scientific knowledge of the role of pollinators in supporting ecosystem function, crop production and diversity maintenance, including the diversity of wild crop relatives and pharmaceuticals, is startlingly scarce. For example, an increasing number of studies are finding that pollinators improve production in crop species that were previously thought not to rely on animal pollinators, while the majority of the world's crops have an unknown reliance on animal pollination (Ashworth et al. 2009, Milfont et al. 2013). In addition to the importance of pollinators to animal-pollinated plants, a recent review found that pollinators used pollen from 101 wind-pollinated plant genera from 25 different families, including ten economically important wind-pollinated crop species (Saunders 2018), indicating that animal-mediated pollination may play a substantial role in the pollination and genetic diversity of wind-pollinated plants.

Pollinator importance likely increases at low latitudes because (a) there are more crop species, including those exported to developed nations (e.g. 316 crops in Mexico), (b) subsistence farmers have a greater dependence on free pollination services, and (c) pollinator-dependent crops produce twice as much income per hectare as non-pollinator-dependent crops (Ashworth et al. 2009). Tropical bees have substantial economic value for their role in pollinating tropical crops (Freitas and Paxton 1998, Freitas et al. 1999, van der Sluijs and Vaage 2016) and production of wax and honey (Vit et al. 2012). Decline of pollination services can lead to decreased food provisioning, including loss of nutrient balance and food diversity (Steffan-Dewenter et al. 2005), and long-term decreases in global plant diversity and provisioning of associated ecosystem services, such as water purification, nutrient cycling, and disease control (Balvanera et al. 2006, Fontaine et al. 2006).

As pollination services maintain biodiversity and bees (Superfamily Apoidea) are the most effective pollinators, research efforts advancing knowledge of tropical bee ecology in biodiversity hotspots should be a global priority of science (Bascompte and Jordano 2007, Dixon 2009). While much attention has been devoted to quantifying the patterns of bee abundance and diversity across land use gradients (Kennedy et al. 2013), as well as the implications for pollination services (Ricketts et al. 2008), there is a surprisingly large gap in knowledge surrounding bee abundance and diversity patterns across elevational gradients, especially in the tropics. Mountainous bee communities in temperate areas exhibit a decline in species richness and abundance with increasing elevation, while the mean body size of temperate bees reveals an increase with increasing elevation (Hoiss et al. 2012). Temperate research on bees across elevational gradients also predicts that smaller-bodied bee species may face higher levels of competition if they shift their ranges to higher elevations in response to warming temperatures (Pereboom and Biesmeijer 2003).

Elevational transect work has reported mid-domain effects across a broad range of taxonomic groups (Rodríguez-Castañeda et al. 2010), and tropical bees may be distributed similarly. The mid-domain effect describes elevational gradients in species diversity, where the random-mixing of species distribution ranges within the constraints of a bounded domain results in a higher overlap in species ranges within the center of the domain than at the upper or lower limits (Colwell and Hurtt 1994, Colwell and Lees 2000), and may exhibit a disproportionate amount of pressure on the distribution of species more likely to be constrained by geographic boundaries (Jetz and Rahbek 2002, Colwell et al. 2004). However, bees are included among the few taxa that show reversed latitudinal gradient patterns in species richness, although global distribution data for most bee taxa have yet to be analyzed (Gaston 2000, Bystriakova et al. 2018). For example, the similarly sized countries of the contiguous United States and Brazil are home to 1107 and 1821 bird species, respectively, but are also home to 4000 and 1678 described bee species, respectively (Freitas et al. 2009). Instead, hotspots of bee diversity have been shown at mid-latitudes and arid environments (Michener 1979). There are exceptions, however; the Neotropical bee tribe, Euglossini, and the economically important Pantropical bee tribe, Meliponini, both increase in species richness with decreasing latitude, while the bee family Andrenidae has no described species in some tropical countries, including Costa Rica (Freitas et al. 2009). Additionally, the economically important bee tribe, Bombini, shows a trend of decreasing species richness with decreasing latitude, in contrast to the understudied small carpenter bee tribe, Ceratinini, which exhibits higher diversity at lower latitudes (Hartfelder 2008, Rehan et al. 2010, Faria and Gonçalves 2013). Since bees do not appear to follow typical global distribution patterns (Pereboom and Biesmeijer 2003), their distribution along tropical mountains may also reveal unique, species-specific or tribe-specific patterns.

Elevational gradients have long been used to test ecological theory and are increasingly used to predict species' risk to climate change (Colwell et al. 2008, McCain and Colwell 2011). Accurate estimates of altitudinal distributions are lacking for most tropical insects, but studies conducted have documented narrow elevational ranges (Macedo et al. 2018). While quantifying elevational range sizes of tropical bee species is scientifically and economically important, there is also an urgent need to know how bee species' abundances vary along elevational gradients, as increased pollinator visits results in higher rates of pollen deposition on flowers (Garibaldi et al. 2013). Smaller elevational gradients in the tropics have been studied less because the differences are often assumed to be marginal, however, plant-animal interactions can vary drastically over short distances on tropical mountains (Rodríguez-Castañeda et al. 2010, Sheldon 2019). Studies along full elevation gradients can show the range extent of species, but typically lack the resolution and replication to detect whether species are equally abundant throughout their range. Species that are common or highly abundant only within a narrow portion of their full altitudinal range should be as vulnerable to changing climate as species with a narrow range. Clumped distributions of tropical species within their narrow elevational ranges may result from habitat specialization or biotic interactions at upper or lower boundary limits and highlight a potentially greater vulnerability to climate change if biotic interactions impede climate induced range shifts (HilleRisLambers et al. 2013). Both insect and plant species, which depend on each other in mutualistic relationships, tend to have more narrow elevational ranges compared to vertebrate species, increasing their potential risk to the combined effects of shifting patterns of precipitation and temperature (Deutsch et al. 2008, McCain and Colwell 2011).

Climate change is posited to have a disproportionately greater impact on tropical species, particularly ectotherms (Deutsch et al. 2008), species residing in the Pacific slopes of Mesoamerica (McCain and Colwell 2011), and mutualists (Dunn et al. 2009, Aslan et al. 2013). Thus, bees of Pacific Central America are among the most at-risk taxa, facing threats from climate change that include the decoupling of interaction partners, desiccation, shifting ranges upslope to novel temperature or precipitation regimes and novel communities, and widowhood (Freitas et al. 2009). Because the narrow altitudinal ranges associated with tropical species has been equated with limited thermal tolerance, much climate change research at low latitudes has focused on species tolerance to temperature extremes (García-Robledo et al. 2016). However, habitat specialization or biotic factors such as competition, predation, and the absence of mutualists can further restrict elevational ranges beyond the species' fundamental niche (Ghalambor et al. 2006, Aguirre and Dirzo 2008, HilleRisLambers et al. 2013). Irrespective of a species thermal limits, the upslope and poleward movement of species globally will lead to the creation of novel biotic interactions, communities, habitats, and moisture regimes, or may decouple important biotic interactions, likely posing a greater risk for tropical species (Urban et al. 2012).

While the effects of warming temperatures have received more attention, changing precipitation patterns are expected to result in more extreme drying periods in Central America (McCain and Colwell 2011), posing a considerable threat to montane bee communities when compared to warming temperatures alone. Moisture is one of the most important abiotic factors affecting the life of terrestrial organisms, yet empirical studies documenting species desiccation tolerance are scarce when compared to thermal tolerance (Montejo-Kovacevich et al. 2020); costly humidity loggers and difficulties with obtaining precipitation data both contribute to this discrepancy. While some evidence exists that insects in particular may be better adapted to tolerate prolonged dry periods (Thorat and Nath 2018), more research is needed because high mortality in insects exposed to prolonged desiccation has also been documented (Moriyama and Numata 2006, Bujan et al. 2016). Global climate change predictions include frequent and severe droughts and erratic precipitation patterns, with these impacts expected to be extreme for mountain-dwelling species of the Pacific slopes of Mesoamerica compared to all other regions globally (McCain and Colwell 2011). Insects living in arid environments should be more capable of tolerating dry periods, but desiccation resistance has not been tested for insects living in seasonally-dry environments, despite the fact that these systems are predicted to experience the most drastic changes in precipitation regimes. Unprecedented precipitation patterns coupled with warming temperatures are expected to induce spatial and temporal shifts in pollinator emergence, resulting in phenological mismatches among plants and pollinators (Vanbergen and the Insect Pollinators Initiative 2013, Benadi et al. 2014). Long-term data documenting patterns of seasonal and interannual fluctuations in pollinator abundances in locations where precipitation data is available can reveal which species may be more vulnerable to the direct or indirect impacts of drought (Wolda 1988).

A two-year study of coffee flower visitors on farms ranging from 975-1140 m elevation in Monteverde, CR revealed that several species of Meliponini were frequent visitors to coffee farms below 1000 m elevation, but were never observed to visit coffee flowers on farms above 1050 m elevation (Peters et al. 2010). This observation inspired long-term monitoring of bee populations in the study area beginning in 2012, with the inclusion of citizen scientists and systematic methodology beginning in 2016. Given the important role of Meliponini and other tropical pollinators in the biotic interactions that support ecological functions, ecosystem services, and ultimately the maintenance of tropical biodiversity (Ruiz-Utrilla et al. 2018), understanding how tropical pollinators may respond to changing climatic conditions is imperative in efforts to conserve tropical pollinator species and pollination services. Focusing on 30 bees species from three tribes, I evaluated the degree of elevational specialization within an elevational gradient that included two life zones: A lower zone consisting of tropical dry forest with higher average and maximum temperatures (750-949 m elevation), and an upper zone consisting of tropical premontane forest with lower average and maximum temperatures (950-1150 m elevation, **Figure 1-1**).

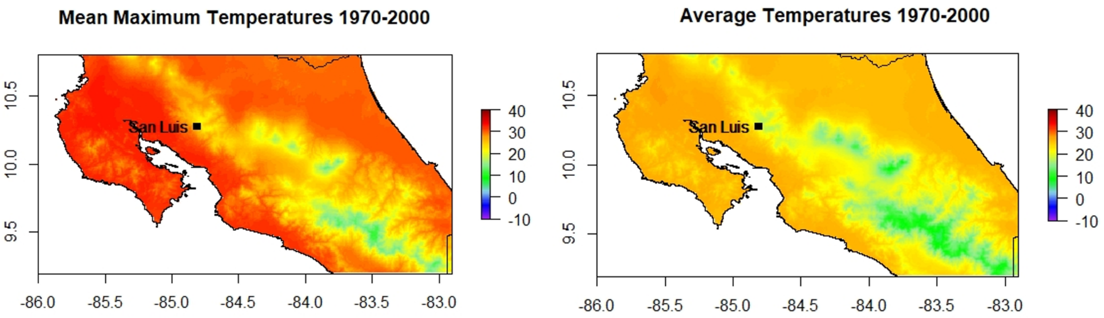


Figure 1-1. Costa Rica maximum and average temperatures (1970-2000). The San Luis study area is indicated on map and is within the hottest and driest region of Costa Rica, the Pacific Northwest.

METHODS

Study Site

Three replicate elevational gradients were established in the San Luis Valley of Monteverde, Puntarenas, Costa Rica (10˚16’ N, 84˚48-49’ W) in 2012 (**Figure 1-2**). The San Luis Valley is located on the Pacific slope of the Tilarán mountain range from 500 to 1200 m above sea-level, with the established transects spanning 750-1150 m above sea-level and positioned approximately 1 km apart. The study area is comprised of tropical dry forest, with low humidity during the dry season (20-60% day time average humidity; Janzen 1988), and pre-montane forest with consistently perhumid conditions (Freiberg 1997), and daily temperatures averaging ~19 ˚C (Harvey 2000). The rainy season lasts from May to November, with mean precipitation of 200-600 mm per month.

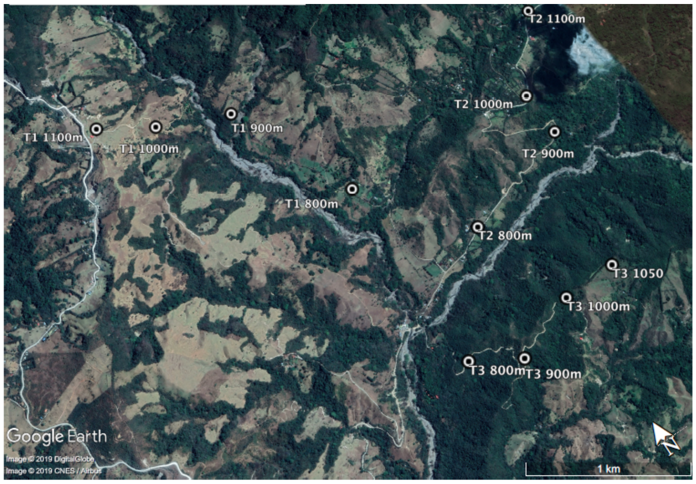


Figure 1-2. Three replicate elevational gradients located in San Luis de Monteverde, Costa Rica. T1: Transect 1; T2: Transect 2; T3: Transect 3. Meters above sea-level are shown for each sampling location. Obtained from Google Maps.

The dry season typically takes place from December to April with a rainfall average of 50-150 mm monthly (Baker 2007, Peters et al. 2010). The region consists primarily of pastures, sugar cane and coffee plantations, and other agricultural plantations subdivided by patches of old primary forest (Peters et al. 2010). A large portion of the study area is protected under conservation easements that preclude forest-clearing activities.

Historical temperature data (1970-2000) in the study area reveals a thermal gradient, with a temperature difference of ~0.7 °C between the 800 m elevation and 1100 m elevation (**Figure 1-3**), with the temperature maximum of the 800 m site an average of 0.71 °C warmer than the 1100 m site, and the temperature average of the 800 m site an average of 0.65 °C warmer that the 1100 m site. Both the 800 m site and the 1100 m site exhibit a similar degree of temperature variation across the year.

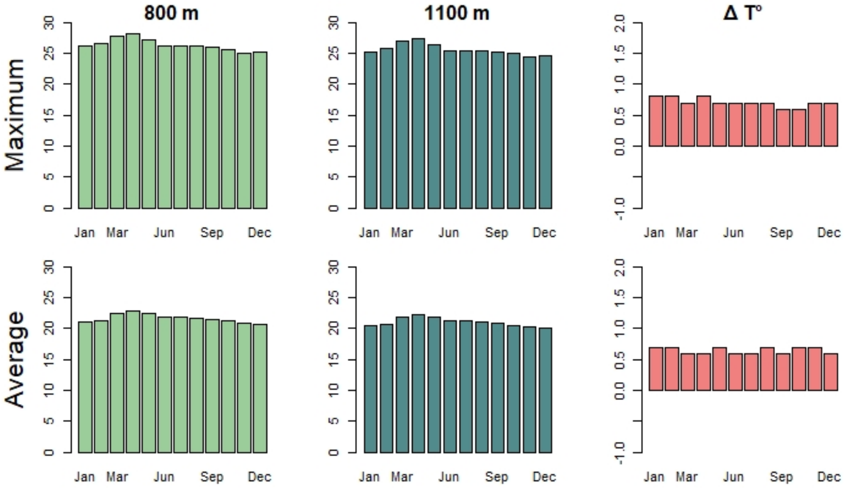


Figure 1-3. Mean of monthly average and maximum temperatures (°C) for 800 and 1100 m a.s.l., and average monthly differences (ΔT°) in San Luis de Monteverde, CR, 1970-2000.

A second study area was established to sample bees at sea-level elevation for a plant-pollinator network study in tropical lowland rain forest (RF). The Osa Peninsula is part of the Isthmian-Pacific moist forest ecoregion and is located at sea-level on the coast of the Pacific Ocean and the Golfo Dulce (**Figure 1-4**). Thirteen sampling sites were established within the tropical countryside of the southeastern corner of the Osa Peninsula, nearby to Puerto Jimenez, CR. Sampling areas consisted primarily of residential and agricultural areas loosely divided by patches of forest (Hunt et al. 2015). Thirty-minute timed-sampling observations of pollinator individuals were conducted at ornamental flowering species at each sampling area during June, July, and December of 2017-2019.

Figure 1-4. Map of Costa Rica detailing location of San Luis de Monteverde, Costa Rica, in relation to a second sampling location near Puerto Jimenez in Osa Peninsula, Costa Rica. Obtained from Google Maps.

Focal Pollinator Tribes

Species belonging to the tribe Ceratinini are highly diverse and globally distributed, consisting of 350 species worldwide, with 15 known species occurring within Costa Rica (Dew et al. 2016). The Ceratinini tribe is one of four belonging to the Apidae subfamily Xylocopinae (Michener 1990a, Rehan et al. 2010). Michener (2007) recognized one genus, *Ceratina*, within this tribe, with 21 subgenera, 16 of which are endemic to the eastern hemisphere and five endemic to the western hemisphere. Species within this tribe are considered to be primitively social due to some feeding behaviors; some females may remain in the nest and assist the mother, acting as drones or foragers, however, no official queen or worker castes have been observed (Eardley and Daly 2007). Smaller holes in the pithy stems of shrubs may indicate nests of species belonging to the Ceratinini tribe, thus earning this tribe the common name of "small carpenter bee." Females can often be seen guarding nest entrances while offspring develop inside the stems (Roubik 1989, Michener 2007). Small carpenter bees do not eat the excavated wood or pith, but instead, use both for partitioning long rows of brood cells. Ceratinini species are considered to be polylectic, as they collect floral rewards from a diversity of unrelated plant species (Raju et al. 2001). Individuals in this tribe range from 2.2-12.5 mm in length (Michener 1990a, 2007).

The bee tribe Meliponini consists of several hundred described species arranged into 21 genera (Michener 1990b), approximately 50 of which occur in Costa Rica (Aguilar et al. 2012). However, most genera have not been sufficiently studied, therefore the actual number of species belonging to this tribe is unknown (Engel 2000). Species within this tribe are known as "social stingless bees" as they have been found to exist in large, perennial, honey-producing colonies with a single queen (Gómez-Escobar et al. 2014). Meliponini species are the primary visitors of many flowering plants in the tropics and are capable of buzz-pollination and nectar-robbing behaviors (Heard 1999). The distribution of species within this tribe is limited to the tropics and subtropics, extending into temperate areas of the southern hemisphere (Kaluza et al. 2018). Meliponini species frequently nest in pithy stems or hollow trees, however, the nest location and external structure are often highly species-specific (Brosi et al. 2008). Social stingless bees exhibit a similar variation in size across species to that of the Ceratinini tribe, ranging from 1.8 to 13.5 mm in length (Michener 2007).

Apis mellifera, the Africanized Honeybee, is a hybrid of European and African Honeybee subspecies whose contemporary distribution spreads through South and Central America and into southern portions of the United States (Sheppard et al. 1991, Calfee et al. 2020). *A. mellifera* are social bees that live in honey-producing colonies consisting of queens, workers, and drones, and may nest in exposed areas or in cavities found in hollow trees or in the ground (Michener 2007). *Apis mellifera* is considered a supergeneralist foraging species and has been documented as the most abundant pollinator visitor to various plant taxa (Howlett et al. 2005, Chaudhary 2006, Davila and Wardle 2008). No known residential apiaries or beekeeping farms exist in the study area.

Pollinator Sampling

Pollinator sampling was conducted during the months of June, July, and December of 2016-2019 from 0800 to 1400 h daily. A combination of passive and active bee collection methods were used, as this is the most effective way to target the entire bee community. Thirty small, plastic 2.5 oz bee bowls painted different colors (florescent yellow, florescent blue, white) using Guerra Paint & Pigment Corp. matte acrylic paint (Droege n.d.), were placed linearly and approximately 5 m apart at each of the sampled elevations (800, 900, 1000, and 1100 m a.s.l.) of each of the three transects. Bowls were placed in order to alternate colors, then filled 2/3 with soapy water. The soap decreases the surface tension such that even small insects are trapped if they land on the surface, and filling the bowls at approximately 2/3 allows for small collisions without spilling the bowl and/ or losing its contents (Droege 2015). Additionally, one yellow and one blue vane trap were each filled to approximately 1/6 of the vane trap container with soapy water and hung from a tree branch for each sample elevation on each transect. Bee bowls and vane traps were deployed 4 times annually per transect from 2016-2018 during the months of June, July, and December, from approximately 0800-1400 h daily, except during periods of heavy precipitation.

A honey spray solution was utilized in order to attract and capture stingless bees from the tribe Meliponini, as bee bowls and vane traps do not effectively capture bees in this tribe (Eltz et al. 2001, Gómez-Escobar et al. 2014). Honey spray solution samples were obtained four times annually per transect during the years 2018-2019 between 800-1400 hours. Vegetation with broad leaves in an open area were located and sprayed with a honey-water solution that is one-part honey and two parts water and marked with flagging tape. Honey sprayed areas were re-sprayed 10-30 minutes following the first application depending on pollinator activity and presence. When honey-sprayed vegetation attracted a high number of bees, all bees on the vegetation were collected using nets or jars during thirty-minute timed-sampling periods.

Hand collection along the three replicate transects was also conducted four times annually per transect in 2016-2018 between 0800 and 1400 h. All flowering trees, shrubs and herbaceous growth along roadsides at each sampled elevation were observed for flower visitors. Bees were collected using hand nets and jars. Thirty-minute timed-observation periods were also conducted at specific flowering plant species that occurred broadly across all elevations along the Main road elevational gradient and at small farms during the years 2017-2019, as well as within the thirteen sampling sites located in the Osa Peninsula during June 2017- July 2019.

Collected bees were preserved using 70% ethanol and exported to Eastern Kentucky University for identification and processing. Meliponini bees were identified to species using identification keys in Michener (2007) and in Jarau and Barth (2008). A Ceratinini bee reference collection was used to identify all Ceratinini bees, and reference bees were identified by Dr. Sandra Rehan.

Environmental Variables

Temperature and precipitation data were acquired from two stand-alone HOBO weather data loggers located at 900 m elevation in the San Luis Valley and at 1140 m elevation at the University of Georgia Costa Rica research station (www.weather.uga.edu). The loggers were placed in open, unshaded areas on relatively even terrain in order to obtain accurate total daily precipitation (mm) and average daily temperature (°C) readings across the elevation gradient. Historical average and maximum temperature data (1970-2000) was obtained from WorldClim version 2.1 (Fick and Hijmans 2017), a high resolution (~ 1 km^2) dataset consisting of spatially interpolated monthly climate data for global land areas obtained from up to 60,000 weather stations globally. Monthly average and maximum temperature data obtained from WorldClim v2 (Fick and Hijmans 2017) were used to visualize historical temperature regimes in the San Luis valley and to determine the historical variation in average and maximum monthly temperatures between 800 m and 1100 m elevation in the study area. Data was extracted and analyzed using the 'raster' and 'maps' packages of R versions 3.1-5 and 3.3.0 (Becker et al. 2018, Hijmans 2020), and visualized using the '*ggplot*' function in the 'ggplot2' package of R version 3.3.0 (Wickham 2016). Precipitation data obtained from on-site HOBO data loggers was used to determine the variation in cumulative rainfall and the onset of the rainy season from 2016-2019.

Statistical Analysis

Bee Bowl Samples Analyses

The response variables of Ceratinini and Apini species abundance in bee bowls were analyzed using linear mixed models (LMM) using the packages 'car' of R version 3.0-8, 'lmerTest' of R version 3.1-2, and 'lme4' of R version 1.1-23 (Kuznetsova et al. 2017, Fox and Weisberg 2018). All models included a fixed effect of elevation and transect ID as the random effect. Likelihood ratio tests were used to assess the significance of the fixed effect of elevation on the response variables (Bolker et al. 2009). The fixed effects of sample year and sample season were also tested for the response variable of Ceratinini species abundance in bee bowls. Ceratinini species were considered to be too infrequently collected if fewer than 15 individuals of that species were collected in bee bowls, as these species made up less than 5% of all Ceratinini species collected in bee bowls from 2016-2018, and, consequently, were excluded from these analyses. Shapiro-Wilk statistic was used to assess distribution normality, and Levene's test was used to assess variance homogeneity (Sileshi 2007).

Honey Sprayed Vegetation Samples Analyses

Since bee bowls do not adequately sample bees from the tribe Meliponini (Gómez-Escobar et al. 2014), species belonging to this tribe were analyzed using data from presence/absence of each species at honey sprayed vegetation using generalized linear mixed models (GLMM) using the packages 'plyr', 'lmerTest', 'lme4', and 'MASS' (Wickham 2011, Venables and Ripley 2013, Kuznetsova et al. 2017). All models included a fixed effect of elevation and transect ID as the random effect. Likelihood ratio tests were used to assess the significance of the fixed effect of elevation on the response variables. Meliponini species were considered to be too infrequently collected if observed in five or fewer honey spray observations, as these species were present in less than 5% of all honey spray observations from 2018-2019. These infrequently collected species were excluded from these analyses.

Thirty-Minute Timed Samples Analyses

Bee presence/absence data obtained during thirty-minute timed-sampling flower observations from 2017-2019 were analyzed using generalized linear models (GLM) using the packages ‘plyr’, ‘lmerTest’, and ‘MASS’ (Wickham 2011, Venables and Ripley 2013, Kuznetsova et al. 2017). Each species was analyzed individually for the effect of elevation category on species presence using a binomial error distribution (Warton et al. 2016). Observations were grouped into 100 m elevation bands and assigned elevation categories as follows: Observations conducted between 750-850 m were categorized in the 800 m elevation band; observations between 851-950 m were categorized in the 900 m elevation band; observations between 951-1050 m were categorized in the 1000 m elevation band; and, observations conducted between 1051-1150 m were categorized into the 1100 m band. Likelihood ratio tests were performed in order to test the significance of the explanatory variable, elevation category, on species presence (Bolker et al. 2009). Species observed in 17 or fewer timed flower samples were considered to be too infrequently collected, as these species were present in less than 5% of all timed flower observations from 2017-2019. These infrequently collected species were excluded from these analyses.

Bee abundance data obtained during thirty-minute timed-sampling flower observations from 2017-2019 were analyzed using linear models (LM) using the package ‘plyr’ (Wickham 2011) to test the effect of elevation category and elevation zone on the response variable of species abundance. Chi-squared goodness of fit tests were used to assess equality in abundances between upper and lower elevation zones

(Bolker et al. 2009). Observations occurring within the 800 m and 900 m elevation categories were classified as the lower zone, and observations occurring within the 1000 m and 1100 m elevation categories classified as the upper zone. Infrequently collected bee species observed in 17 or less timed flower samples were excluded from these analyses.

Bee Community Composition and Similarity Analyses

A constrained ordination was conducted using a canonical correspondence analysis (CCA) of Ceratinini, Meliponini, and Apini species composition at each sampled elevation on each of the 3 elevational transects using the '*cca*' function available in the 'vegan' package of R version 2.5-6 (Oksanen et al. 2013), using quarter power transformation of a species matrix in order to reduce the effect that large variances in species abundances have on analysis, with singletons (n = 2) and doubletons (n = 1) removed. The results of a CCA can reveal whether the explanatory variables of transect (site) and elevation had a significant impact on the variation in bee community composition (Buttigieg and Ramette 2014). Biplot arrows represent explanatory variables and species belonging to the tribes Meliponini, Ceratinini, and Apini are represented by points accompanied with abbreviated species names. In order to compare the bee community composition of each sampled elevation, similarity of community composition among the elevation categories was analyzed using the *'SimilarityMult'* function in the 'Spade-R' package of R version 0.1.1 (Chao et al. 2016), using the Morisita index to conduct multi-stage community comparisons with singletons (n = 4) removed. This approach is the most robust method for comparing community overlap as it allows for concurrent analysis of several samples and among communities with a species overlap greater than two (Chao et al. 2008). Communities were considered to be statistically different if similarity values were less than 0.19 (Rodríguez-Castañeda et al. 2010).

RESULTS

A total of 967 samples were collected from the three elevational gradients during the four years of the study (2016-2019). The samples included 144 bee bowl samples, 69 vane trap samples, and 111 hand collected samples during 2016 to 2018; 524 samples from timed observations at flowering plants during 2017 to 2019; and 117 honey spray solution samples during 2018 to 2019. A total of 11,753 bees representing 30 species from the tribes Ceratinini, Meliponini and Apini were collected (**Table 1-1**). Eighteen of the 30 species were also collected from the second sampling location at 0 m elevation in the Osa Peninsula. Of the individuals collected from the elevational gradients in the San Luis Valley, 3825 individuals were collected from bee bowl samples, 28 individuals were collected from vane trap samples, 1051 individuals were hand-collected from flowers during hand collection samples, 2869 individuals were collected from flowers during thirty-minute timed flower samples, and 3980 individuals were collected from honey sprayed vegetation samples.

Table 1-1. Abundance of bee species collected in each elevation category across the replicate elevational gradients in the San Luis Valley and from 0 m in the Osa Peninsula, 2016-2019.

Bee Species (Abbreviation)	0 m	800 m	900 m	1000 m	1100 m
Apis mellifera (AM)	545	360	236	363	305
Ceratina auriviridi (CA)	0	3	0	2	0
Ceratina buscki (CB)	1	21	13	10	3
Ceratina chloris (CH)	251	1	0	0	0
Ceratina cobaltina (CC)	0	4	7	6	2
Ceratina dimidiata (CD)	0	2	5	5	4
Ceratina eximia (CE)	1	27	22	21	2
Ceratina ignara (CI)	0	8	5	3	4

Table 1-1 (continued)

Bee Species (Abbreviation)	0 m	800 m	900 m	1000 m	1100 m
Ceratina rectangulifera (CR)	0	869	1302	1045	430
Ceratina trimaculata (CT)	1	34	87	159	167
Ceratina zeteki (CK)	0	0	0	0	1
Cephalotrigona zexmeniae (CZ)	4	7	50	1	0
Melipona beechii (MB)	0	0	1	5	1
Melipona fallax (MF)	0	4	4	2	27
Melipona fasciata (MA)	163	6	20	0	6
Nannotrigona mellaria (NM)	107	19	16	0	0
Oxytrigona isthmina (OI)	0	10	0	1	0
Partamona orizabaensis (PO)	198	294	153	82	669
Plebeia frontalis (PF)	5	22	156	75	6
Plebeia pulchra (PP)	0	39	179	236	110
Scaptotrigona mexicana (SM)	0	26	367	62	105
Scaptotrigona pectoralis (SP)	7	2	17	0	1
Tetragona dorsalis (TD)	72	65	21	64	5
Tetragona perangulata (TP)	53	0	0	1	0
Tetragonisca angustula (TA)	0	323	278	167	82
Trigona corvina (TC)	423	140	232	376	167
Trigona fulviventris (TF)	1298	168	154	361	473
Trigona fuscipennis (TU)	271	29	21	13	0
Trigona silvestriana (TS)	27	121	12	28	1
Trigonisca buyssoni (TB)	3	31	81	15	5

Across the three elevational gradients, a total of 2635 individuals of the three tribes were collected near 800 m elevation, a total of 3439 individuals were collected near 900 m elevation, a total of 3103 individuals were collected near 1000 m elevation, and 2576 individuals were collected near 1100 m elevation. A total of 4273 individuals were Ceratinini bees, 6216 were Meliponini bees, and 1264 individuals *Apis mellifera,* the only bee species in the study area that belongs to the tribe Apini.

Bee Abundance in Bee Bowls

A total of 3800 Ceratinini bees were collected in bee bowls during 2016-2018. The most abundant species collected in bee bowls was *Ceratina rectangulifera* (3350 individuals), and the second most abundant species was *Ceratina trimaculata* (422 individuals). No other Ceratinini bee species was collected in sufficient abundance using bee bowls to statistically test differences in their abundance between elevations. The abundance of *C. rectangulifera* ($X^2_6 = 9.01$; $p = 0.029$) differed across elevations while the abundance of *C. trimaculata* ($X^2_6 = 7.73$; $p = 0.052$) did not differ across elevations (**Table 1-2**).

Ceratina rectangulifera abundance was lowest at 1100 m compared to the other three elevations, while *C. trimaculata* had a significantly higher abundance at 1000 m and 1100 m elevation when compared to 800 m elevation ($X^2 = 157.89$; $p = <0.001$; **Figure 1-5**). Season of collection also had a significant effect on total Ceratinini abundance in bee bowls (i.e., the two species combined; $X^2 = 3346.4$; $p = <0.001$). On average, fewer individuals were collected during December samples (13 ± 3.92) compared to June-July samples (205 ± 45.61); (**Figure 1-6**).

Table 1-2. Results of generalized mixed model analyses testing for the effect of elevation on the abundance of *C. rectangulifera* and *C. trimaculata* across replicate transects, 2016-2018. Significance assessed using likelihood ratio tests.

Species	Model	Degrees of freedom	AIC Score
CR	~1+(1|Transect)	3	26.01
	(Elevation)+(1|Transect)	6	22.997
CT	~1+(1|Transect)	3	43.554
	(Elevation)+(1|Transect)	6	41.826

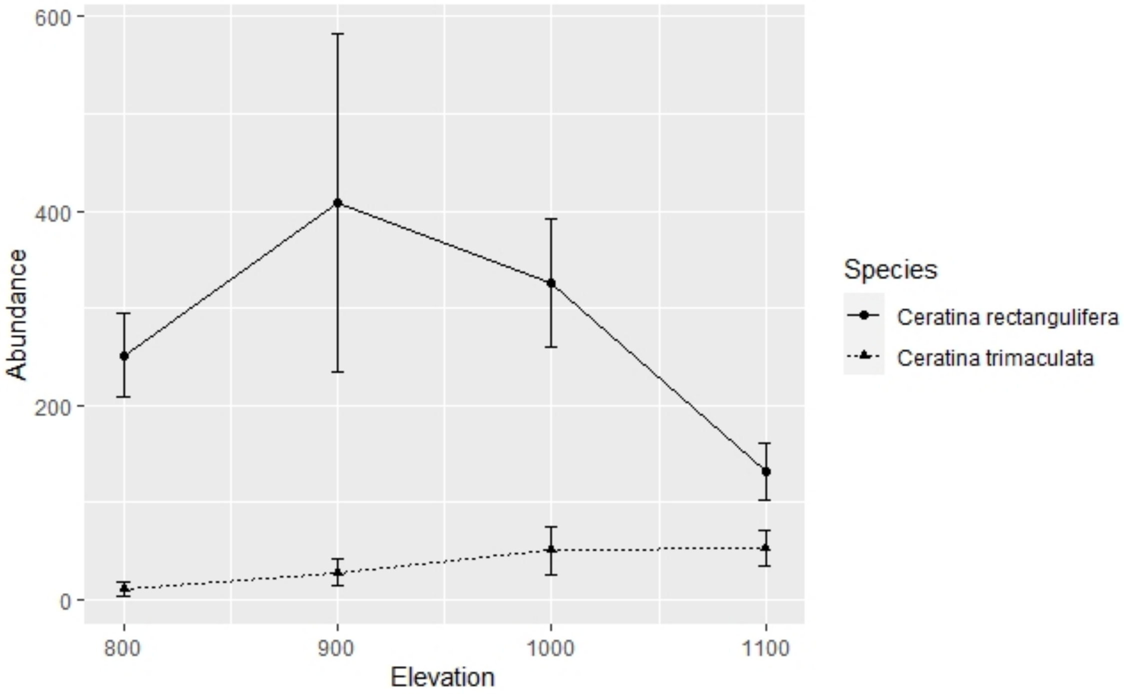


Figure 1-5. Average abundances across three replicate transects of *Ceratina rectangulifera* and *Ceratina trimaculata* individuals obtained from bee bowls at each sampled elevation, 2016-2018.

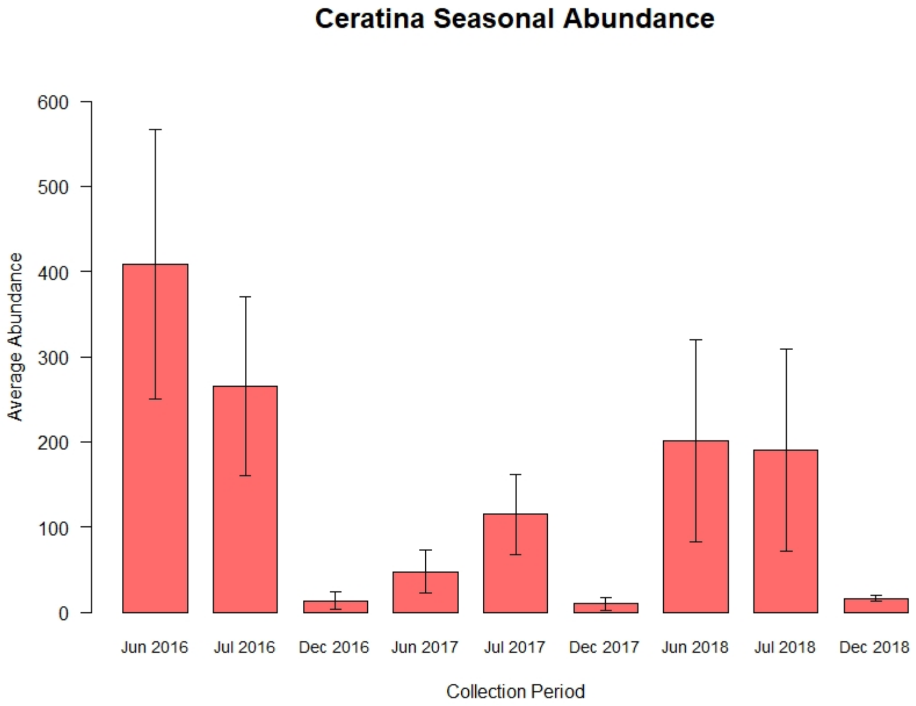
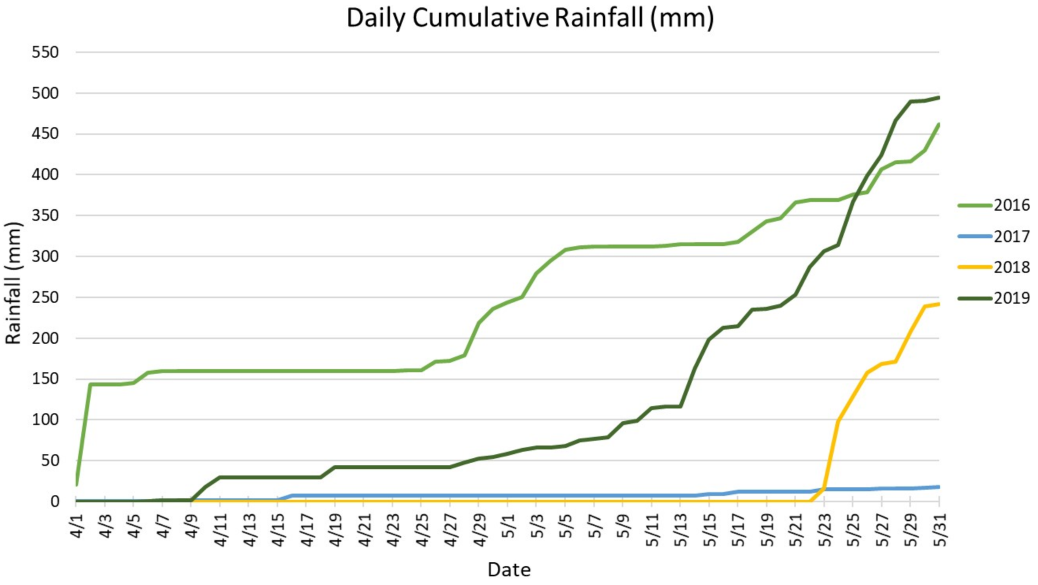


Figure 1-6. Seasonal variation of the average abundances of individuals collected in bee bowls belonging to the tribe Ceratinini in June, July, and December, 2016-2018.

Significantly fewer individuals were also collected in 2017 compared to 2016, with intermediate numbers of individuals collected in 2018 ($X^2 = 943.18$; $p = <0.001$). While I don't have enough years of data to draw conclusions, rainfall data show that there was a drought during the onset of the rainy season in 2017 with only ~18 mm of precipitation from 1 April- 31 May 2017 (**Figure 1-7**). In 2018 there was a delay in the onset of the rainy season.



Year	Cumulative Rainfall (mm)	Number of Rainy Days
2016	462.026	35
2017	18.034	14
2018	241.808	9
2019	494.284	39

Figure 1-7. Variation in cumulative rainfall and the number of rainy days observed during the onset of the rainy season, April-May, 2016-2019.

Bee Presence/ Absence in Honey Spray

A total of 16 Meliponini species were collected during honey sprayed vegetation sampling. Of these, six species were observed in five or fewer honey spray observations and were, therefore, considered to be too infrequently collected to statistically test (**Table 1-3**). Infrequently collected bees included *M. beechii* and *M. fallax,* which were only observed in the upper elevation zone, above 1000 m elevation. Other infrequently collected bees, *M. fasciata, N. mellaria*, and *T. fuscipennis,* were only observed in the lower elevation zone, < 950 m elevation. The species present in the highest number of samples were *T. angustula*, followed by *P. pulchra*, *P. orizabaensis*, *P. frontalis*, *T.*

fulviventris, *T. corvina*, *S. mexicana*, *T. buyssoni*, *T. dorsalis*, and *T. silvestriana*. The presence of *P. frontalis* ($X^2_3 = 9.77$; p = 0.001), *T. dorsalis* ($X^2_3 = 4.81$; p = 0.028), *T. angustula* ($X^2_3 = 5.77$; p = 0.016), *T. fulviventris* ($X^2_3 = 5.25$; p = 0.021), and *T. silvestriana* ($X^2_3 = 16.09$; p = <0.001) differed across elevations (**Table 1-4**). The presence of *P. orizabaensis* ($X^2_3 = 0.16$; p = 0.686), *P. pulchra* ($X^2_3 = 6.19$; p = 0.103), *S. mexicana* ($X^2_3 = 1.73$; p = 0.189), *T. corvina* ($X^2_3 = 0.008$; p = 0.930), and *T. buyssoni* ($X^2_3 = 0.065$; p = 0.982) did not differ among elevations.

Table 1-3. Proportion of honey spray samples containing Meliponini observations at each elevation in 2018-2019. Species present in five or fewer honey spray observations were excluded from statistical analyses and are in bold. Species found to have a statistically significant relationship between presence and elevation are indicated with an asterisk.

Species	800 m	900 m	1000 m	1100 m	Total Proportion
MB	0.000	0.000	0.029	0.000	0.009
MF	0.000	0.000	0.029	0.036	0.017
MA	0.042	0.033	0.000	0.000	0.017
NM	0.083	0.067	0.000	0.000	0.034
PO	0.375	0.133	0.176	0.357	0.248
PF*	0.208	0.633	0.088	0.036	0.239
PP	0.250	0.500	0.412	0.250	0.359
SM	0.083	0.167	0.118	0.214	0.145
SP	0.042	0.067	0.000	0.036	0.034
TD*	0.167	0.100	0.000	0.036	0.068
TA*	0.625	0.467	0.500	0.214	0.444
TC	0.208	0.133	0.176	0.143	0.162
TF*	0.458	0.167	0.088	0.179	0.205
TU	0.042	0.133	0.000	0.000	0.043
TS*	0.292	0.000	0.029	0.000	0.068
TB	0.208	0.200	0.059	0.071	0.128

Table 1-4. Results of binomial generalized mixed model analyses testing for the effect of elevation on the presence of Meliponini species present in greater than five honey spray observations, 2018-2019. Species found to have a statistically significant relationship between presence and elevation are indicated with an asterisk.

Species	Model	Degrees of freedom	AIC Score
PO	~1+(1|Transect)	2	123.41
	~Elevation+(1|Transect)	3	125.42
PF*	~1+(1|Transect)	2	121.58
	~Elevation+(1|Transect)	3	113.81
PP	~1+(1|Transect)	2	138.04
	~Elevation+(1|Transect)	3	140.04
SM	~1+(1|Transect)	2	95.55
	~Elevation+(1|Transect)	3	95.82
TD*	~1+(1|Transect)	2	59.59
	~Elevation+(1|Transect)	3	56.78
TA*	~1+(1|Transect)	2	143.93
	~Elevation+(1|Transect)	3	140.16
TB	~1+(1|Transect)	2	84.26
	~Elevation+(1|Transect)	3	82.85
TC	~1+(1|Transect)	2	70.38
	~Elevation+(1|Transect)	3	72.38
TF*	~1+(1|Transect)	2	114.76
	~Elevation+(1|Transect)	3	111.51
TS*	~1+(1|Transect)	2	57.31
	~Elevation+(1|Transect)	3	43.22

Bee Presence/ Absence in Timed Flower Observations

A total of 26 bee species from the three focal tribes were collected from flowers during thirty-minute timed observations in 2017-2019. Of these, 15 species were observed in 17 or less timed flower observations and were, therefore, considered to be too infrequently collected to statistically test (**Table 1-5**). Infrequently collected bee species *C. chloris, M. beechii,* and *S. pectoralis*, were each collected in only one timed flower observation.

Table 1-5. Proportion of timed flower observations at each elevation in 2017-2019. Species present in 17 or fewer timed flower observations were excluded from statistical analyses and are in bold. Species found to have a statistically significant relationship among presence and elevation are indicated with an asterisk.

Species	800	900	1000	1100	Total Proportion
AM	0.160	0.175	0.122	0.229	0.177
CA	0.010	0.000	0.008	0.000	0.004
CB	0.060	0.026	0.053	0.006	0.032
CH	0.010	0.000	0.000	0.000	0.002
CC	0.010	0.044	0.038	0.011	0.025
CD	0.010	0.018	0.015	0.011	0.013
CE	0.040	0.018	0.069	0.006	0.031
CR*	0.210	0.079	0.130	0.034	0.101
CT	0.000	0.009	0.023	0.028	0.017
CZ	0.030	0.026	0.008	0.000	0.013
MB	0.000	0.000	0.008	0.000	0.002
MF	0.000	0.000	0.008	0.034	0.013
MA	0.000	0.026	0.000	0.011	0.010
NM	0.030	0.026	0.000	0.000	0.011
PO	0.140	0.167	0.069	0.229	0.158
PF*	0.050	0.158	0.046	0.000	0.055
PP*	0.130	0.149	0.137	0.034	0.103
SM	0.050	0.061	0.031	0.067	0.053
SP	0.010	0.000	0.000	0.000	0.002
TD*	0.140	0.061	0.153	0.017	0.084
TA*	0.240	0.272	0.153	0.028	0.153
TC*	0.160	0.105	0.084	0.017	0.080
TF*	0.230	0.386	0.496	0.525	0.431
TU	0.060	0.026	0.015	0.000	0.021
TS*	0.190	0.026	0.038	0.006	0.053
TB	0.070	0.044	0.031	0.000	0.031

Ceratina chloris, N. mellaria, and *S. pectoralis* were only observed in the lower elevation zone, while *M. beechii* and *M. fallax* were only observed at the upper elevation zone. *Cephalotrigona zexmeniae, T. buyssoni,* and *T. fuscipennis* were observed at all elevations except for 1100 m, while *C. trimaculata* was observed at all elevations except for 800 m. All other infrequently collected bee species were observed in both elevation zones or at all elevations. The most frequently collected species included *A. mellifera*, *C. rectangulifera*, *P. orizabaensis*, *P. frontalis*, *P. pulchra*, *S. mexicana*, *T. dorsalis*, *T. angustula*, *T. corvina*, *T. fulviventris*, and *T. silvestriana*.

The presence of *C. rectangulifera* ($X^2_2 = 16.32$; p = <0.001), *P. frontalis* ($X^2_2 = 12.08$; p = <0.001), *P. pulchra* ($X^2_2 = 8.63$; p = 0.003), *T. dorsalis* ($X^2_2 = 8.27$; p = 0.004), *T. angustula* ($X^2_2 = 34.19$; p = <0.001), *T. corvina* ($X^2_2 = 18.69$; p = <0.001), *T. fulviventris* ($X^2_2 = 29.69$; p = <0.001), and *T. silvestriana* ($X^2_2 = 33.75$; p = <0.001) differed across elevations (**Table 1-6; Figure 1-8**), while the presence of *A. mellifera* ($X^2_2 = 1.98$; p = 0.160), *P. orizabaensis* ($X^2_2 = 2.99$; p = 0.083), and *S. mexicana* ($X^2_2 = 0.17$; p = 0.681) were not significantly different among elevations.

Table 1-6. Results of binomial generalized mixed model analyses testing for the effect of elevation on the presence of bee species present in greater than 17 timed flower observations, 2017-2019. Species found to have a statistically significant relationship between presence and elevation are indicated with an asterisk.

Species	Model	Degrees of Freedom	AIC Score
AM	Presence~1	1	454.91
	Presence~ElevationCAT	2	454.94
CR*	Presence~1	1	325.18
	Presence~ElevationCAT	2	310.85
PO	Presence~1	1	427.29
	Presence~ElevationCAT	2	426.29
PF*	Presence~1	1	215.5
	Presence~ElevationCAT	2	205.42
PP*	Presence~1	1	329.12
	Presence~ElevationCAT	2	322.49
SM	Presence~1	1	210.17
	Presence~ElevationCAT	2	212

Table 1-6 (continued)

Species	Model	Degrees of Freedom	AIC Score
TD*	Presence~1	1	287.65
	Presence~ElevationCAT	2	281.38
TA*	Presence~1	1	418.44
	Presence~ElevationCAT	2	386.24
TC*	Presence~1	1	278.78
	Presence~ElevationCAT	2	262.09
TF*	Presence~1	1	608.75
	Presence~ElevationCAT	2	581.06
TS*	Presence~1	1	210.17
	Presence~ElevationCAT	2	178.42

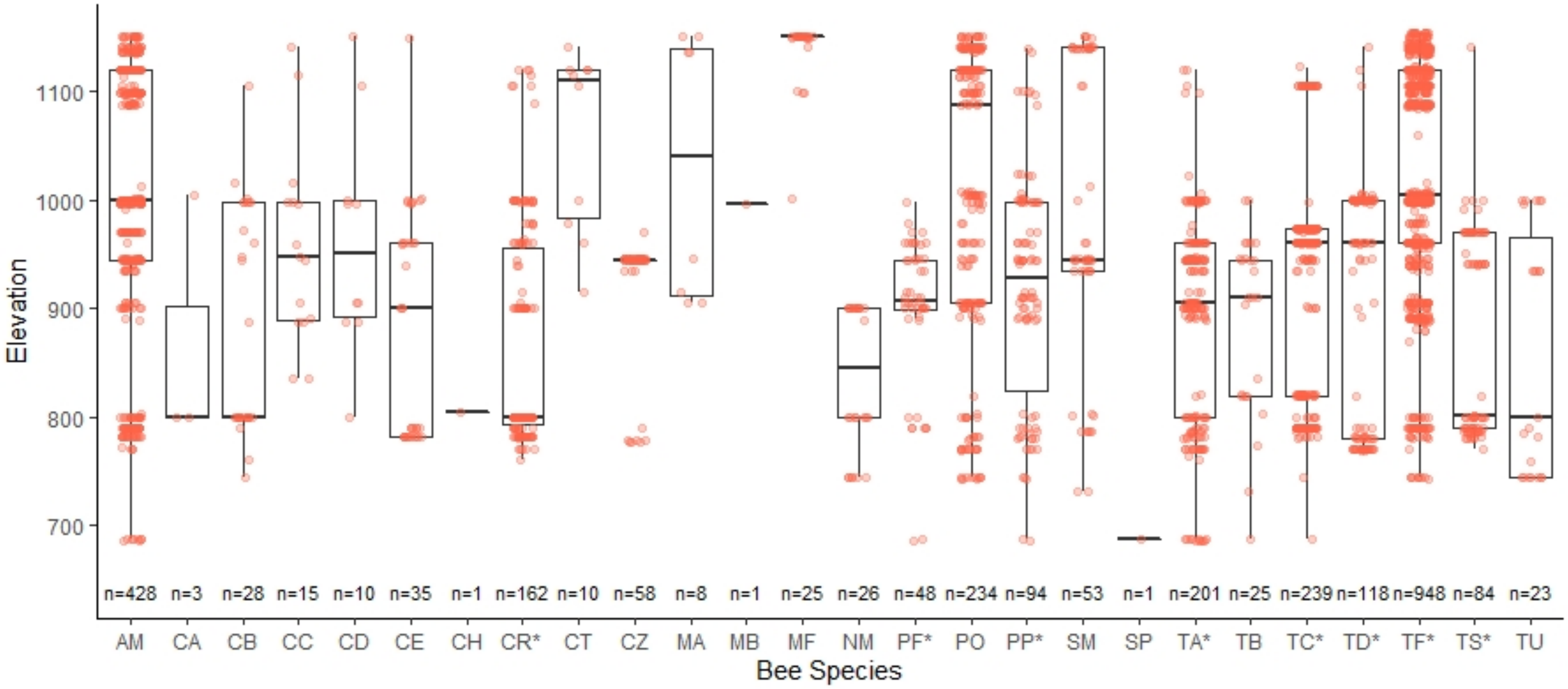


Figure 1-8. A subset of 2878 bees representing three tribes from a total of 3919 bees that were collected directly from flowering plant species during 30-minute timed observations. Species present in 17 or fewer samples were excluded from statistical analysis. An asterisk denotes a statistically significant effect of elevation on bee species presence using a binomial glm ($p < 0.05$). Each red dot is one individual.

Bee Abundance in Timed Flower Observations

A total of 2878 Ceratinini, Meliponini, and Apini bees were collected during thirty-minute timed flower observations in 2017-2019. Fifteen bee species were observed in 17 or fewer samples and were considered to be too infrequently collected to statistically test. Abundances of *A. mellifera* ($X^2_1 = 1.45.75$; $p = <0.001$), *C. rectangulifera* ($X^2_1 = 39.51$; $p = <0.001$), *P. orizabaensis* ($X^2_1 = 30.15$; $p = <0.001$), *P. frontalis* ($X^2_1 = 14.08$; $p = <0.001$), *P. pulchra* ($X^2_1 = 6.13$; $p = 0.013$), *T. angustula* ($X^2_1 = 46.8$; $p = <0.001$), *T. fulviventris* ($X^2_1 = 410.73$; $p = <0.001$), and *T. silvestriana* ($X^2_1 = 9.33$; $p = 0.002$) significantly differed among upper and lower elevation zones (**Table 1-7**). The abundances of *S. mexicana* ($X^2_1 = 0.17$; $p = 0.68$), *T. dorsalis* ($X^2_1 = 1.66$; $p = 0.198$), and *T. corvina* ($X^2_1 = 2.62$; $p = 0.106$) were not significantly different among elevation zones. Only the abundance of *T. fulviventris* ($F_{1,2} = 30.17$; $p = 0.032$) significantly differed across elevation categories, however, the abundance analyses did not include observations when the bee species was not observed, i.e. timed flower samples where bee abundance was zero.

Table 1-7. Linear model results for the effect of elevation category and elevation zone on frequently collected Apini, Ceratinini, and Meliponini species frequencies in thirty-minute timed flower observations, 2017-2019. Significance assessed using Chi-square goodness-of-fit tests.

Species	Model	Degrees of Freedom	P-value
AM	freq~ElevationCAT	2	0.087
	freq~Zone	1	<0.001
CR	freq~ElevationCAT	2	0.147
	freq~Zone	1	<0.001

Table 1-7 (continued)

Species	Model	Degrees of Freedom	P-value
PO	freq~ElevationCAT	2	0.226
	freq~Zone	1	<0.001
PF	freq~ElevationCAT	2	0.939
	freq~Zone	1	<0.001
PP	freq~ElevationCAT	2	0.362
	freq~Zone	1	0.013
SM	freq~ElevationCAT	2	0.682
	freq~Zone	1	0.68
TD	freq~ElevationCAT	2	0.696
	freq~Zone	1	0.198
TA	freq~ElevationCAT	2	0.221
	freq~Zone	1	<0.001
TC	freq~ElevationCAT	2	0.709
	freq~Zone	1	0.106
TF	freq~ElevationCAT	2	0.032
	freq~Zone	1	<0.001
TS	freq~ElevationCAT	2	0.212
	freq~Zone	1	0.002

Bee Community Similarity and Composition

A total of 5678 bees belonging to 24 species were used in the constrained ordination. Samples used in this analysis included all Apini, Ceratinini, and Meliponini samples obtained using bee bowls, honey sprayed vegetation, vane traps, and hand collection sampling on all three transects during 2016-2018. The effects of elevation and transect ID explained 41.7% of the variation in bee community composition across the elevation gradient ($F_{pseudo3,8} = 1.91$, $p = 0.002$; **Figure 1-9**). Elevation and transect ID each explained ~21% of the variation in bee community composition (20.9%; $F_{pseudo(1,8)} = 2.52$, $p = 0.004$; 20.8%; $F_{pseudo(2,8)} = 1.60$, $p = 0.037$). Bee species associated with higher elevations included *T. corvina*, *T. dorsalis*, *T. fulviventris*, *C. trimaculata*, and *P. pulchra*.

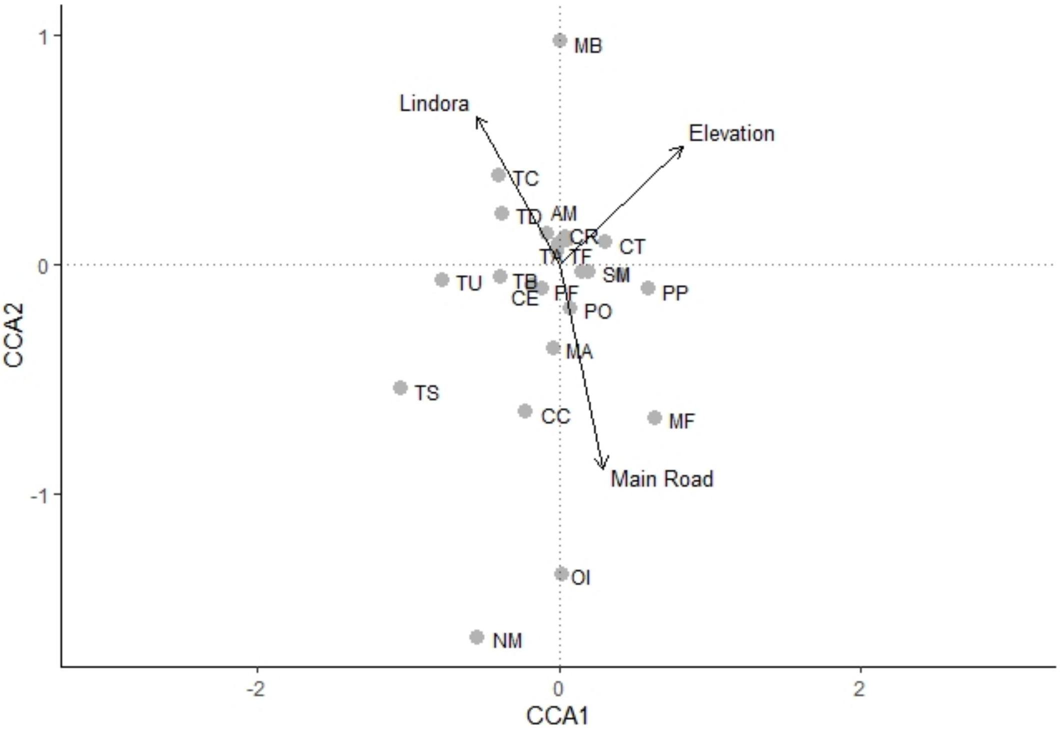


Figure 1-9. Canonical correspondence analysis ordination revealing elevation and site to be significant in explaining ~42% of the variation in bee community composition across an elevation gradient, 2016-2018.

Bee species associated with lower elevations included *C. rectangulifera*, *T. silvestriana*, *P. frontalis*, and *T. angustula*.

Multiple stage similarity comparisons were conducted on the entirety of Apini, Ceratinini, and Meliponini samples collected from 2016-2019. Similarity comparisons demonstrated high community similarity values and low turnover among the elevation categories (**Table 1-8).** Bee communities at 800 m and 900 m exhibit the highest degree of similarity in my system (mean Morisita similarity value *c*. 0.989), while bee communities at 900 m and 1100 m exhibit the highest degree of dissimilarity (mean Morisita similarity value *c*. 0.836). The community present within the 1100 m elevation category exhibited the highest degree of dissimilarity with all other elevation categories.

Table 1-8. Community similarity matrix including Apini, Ceratinini, and Meliponini individuals observed in all sampling methods, singletons removed, 2016-2019.

Elevation Category	800 m	900 m	1000 m	1100 m
800 m	1.000	0.989	0.987	0.871
900 m	---	1.000	0.977	0.836
1000 m	---	---	1.000	0.930
1100 m	---	---	---	1.000

DISCUSSION

Quantifying the vulnerability of pollination services to global climate change in Neotropical montane regions will require a better understanding of how the primary service providers are distributed altitudinally, as well as the driving factors behind their altitudinal distributions (Chen et al. 2009b, Rodríguez-Castañeda et al. 2010, Hoiss et al. 2015). Focusing on a subset of the most commonly collected bee species from the Pacific slopes of Costa Rica, I report that the presence of 9 of 26 bee species were significantly different by elevation, and 14 of the remaining 17 were collected too infrequently to statistically estimate differences among elevation. The observed elevational specialization could be due to biotic factors, such as mutualistic relationships (Olesen and Jordano 2002, Benadi et al. 2014) and competition for floral resources (Hoiss et al. 2012, Chan et al. 2019), or abiotic factors, such as thermal or desiccation tolerances (Longino and Colwell 2011, Theobald et al. 2016, Coutinho et al. 2018).

In this study I did not directly test bee species for their thermal limits, however, my results suggest that a combination of thermal tolerance and biotic factors may account for the observed patterns of elevational specialization. For example, the two species that were most commonly collected in bee bowls, *Ceratina rectangulifera* and *C. trimaculata*, were observed at all elevations across the elevational gradient studied, but C. *rectangulifera*, although more common overall, was observed more frequently in the lower zone, while C. *trimaculata* was observed more frequently in the upper zone. *Ceratina rectangulifera* and *trimaculata* are species similar in body size, color, floral resource use, and nesting habitat (Michener 2007, Engel 2015, Rehan et al. 2015), yet these species exhibit dissimilar elevational specialization, suggesting spatial niche partitioning potentially as a result of interspecific interference competition (Gottlieb et al. 2005, Gonzalez et al. 2020). Patterns of several other bee species analyzed in this study suggest that the species are lower elevation specialists. For example, *C. chloris*, *N. mellaria*, *T. perangulata*, and *C. zexmeniae* were only infrequently observed in the lower elevation zone but were also observed frequently near sea-level at the second study site in the Osa Peninsula. One stingless bee species found to be a lower zone specialist in our study area, *T. angustula*, is kept throughout Costa Rica because its honey has medicinal properties (Aguilar et al. 2012). Because of its economic importance, one study evaluated the hive's productivity along an elevational gradient, and found a relationship between productivity and temperature (Brosi 2009). Farmers in the study area have also attempted to keep hives in boxes unsuccessfully above 1100 m elevation. My study indicates that the distribution of *T. angustula* across the elevational gradient will expand at higher elevations and contract at lower elevations in response to climate change scenarios. This could potentially supplement the possible reduction in pollination services at higher elevations that may result from the loss of high-elevation bee species in response to projected climate change scenarios. Finally, a few bee species, such as *A. mellifera*, *T. fulviventris*, and *S. mexicana*, did not show elevational specialization, and evidence supports that these bee species are both generalists in resource use and have broad thermal tolerances (Roubik 1981, Martínez-Hernández et al. 1994, Atmowidjojo et al. 1997, Pinkus-Rendon et al. 2005). Tropical montane pollination services may be robust to climate change if services provided by these generalists are as effective as the entire assemblage.

Because the narrow altitudinal ranges associated with tropical species has been equated with limited thermal tolerance, much climate change research at low latitudes has focused on species tolerance to temperature extremes (García-Robledo et al. 2016). However, habitat specialization or biotic factors such as competition, predation and the absence of mutualists can further restrict elevational ranges beyond the species' fundamental niche (Ghalambor et al. 2006, HilleRisLambers et al. 2013, Aguirre-Jaimes et al. 2018). A more comprehensive approach to quantifying species response to climate change therefore should evaluate non-climatic and climatic factors influencing species elevational distributions. Irrespective of a species thermal limits, the upslope and poleward movement of species globally will lead to the creation of novel biotic interactions, communities, habitats, and moisture regimes or may decouple important biotic interactions, likely posing a greater risk for tropical species (Urban et al. 2012).

Studies from temperate mountain dwelling pollinator communities have predicted that species will expand their ranges at the cool, upper elevational limits and contract their ranges at the warm, lower elevational limits, creating novel communities where competition may lead to local extirpation or extinction (Murray et al. 2009, Kocsis and Hufnagel 2011). If bee species in my study that were more frequently observed in the lower elevation zone (<1000 m), such as *P. frontalis*, *T. silvestriana*, and *C. rectangulifera*, shift their ranges 600 m upslope, as predicted for all biota in the tropics (Colwell et al. 2008), these species could potentially be confronted with competing species such as *M. beechii*, *M. fallax*, and *C. trimaculata*, species that were more frequently observed in the upper elevation zone. These upper zone specialists, however, will be confronted with the disappearance of their thermal habitat on short mountains, such as the Monteverde mountain in the Tilarán Cordillera (1800 m highest peak; the location of this study) if they shift their ranges 600 m upslope as predicted. Most research aimed at quantifying altitudinal distributions of species in Costa Rica has been conducted on the two mountain ranges in the southern half of the country, which have much taller peaks (>2500 m elevation) compared to the two mountain ranges in the northern half of the country, the Guanacaste and Tilarán Cordilleras, that are comprised of relatively short mountains with the highest peaks from 1500-2000 m elevation (Barrantes 2009). In my study, lower zone bee species with distributions of 800-1000 m elevation will be the species predicted to replace species at the top of short mountains (1400-1600 m elevation), while upper zone species that I only observed around 1100 m elevation are species that are predicted to move off the top (Sheldon 2019). Several of the upper zone specialists, including *M. beechii* and *M. fallax*, are economically important stingless bee species that were traditionally kept throughout Central America for honey, but are now considered to be rare species throughout much of their previous range (Aguilar et al. 2012). Future work should aim to simulate how these novel bee communities and extirpations might impact local food security as well as cash crops.

My study spanned multiple years, with a total of 264 hours collecting bees directly from flowers, as well as 37 days trapping bees in bee bowls, collecting bees from roadside and farm vegetation, vane traps, and honey spray samples. After extensive sampling, fourteen bee species from my focal tribes were collected too infrequently during timed observations to statistically analyze differences in their presences across the elevation gradient. Rare bee species, as well as small-bodied species with peak abundances in narrow elevation ranges, both have high extinction risk, as smaller-bodied bees have been documented as easily displaced when bee communities also include larger bees (Frankie et al. 1997), and rare species can be displaced by common species due to overlap in resource use (Steffan-Dewenter and Tscharntke 2000), suggesting that small or uncommon bee species may exhibit species-specific responses to changes in temperature and thermal limits that may be significantly defined by species morphological traits or interspecific competition (Cane and Neff 2011, Gonzalez et al. 2020). An alternative explanation for the infrequency of these particular bee species in my dataset may be that I primarily sampled from treelets, shrubs and herbaceous plants (Cane et al. 2000, Roulston et al. 2007), and I did not sample sufficiently from the preferred plant species of these bee species. Additionally, this study takes place on a narrow elevational gradient, which may under sample bee species with elevational ranges occurring above or below the study area.

The significant relationship observed among the pollinator community and elevation, coupled with bee sensitivity to changing and increasing temperatures (Dixon et al. 2009, Hoiss et al. 2012), suggests species distributions and elevation specialization may, at least in part, be driven by changes and increases in temperature as a result of climate warming (Parmesan and Yohe 2003, García-Robledo et al. 2016). Additionally, ectotherms exhibit low thermal tolerance plasticity (Gunderson and Stillman 2015), although species may compensate for this by shifting active foraging times to cooler parts of the day or seeking out cooler microclimates (Sheldon 2019). Precipitation patterns and rainfall gradients may also have an effect on bee assemblages and distribution patterns (Wolda 1988). The observed abundances of *Ceratina* *rectangulifera* and *trimaculata* appear to exhibit interannual fluctuations reflecting seasonal rainfall patterns in the study area, with low abundances observed during the dry season and high abundances during the rainy season. The onset of the rainy season in the study area falls between April and May of each year, with annual variations occurring typically in response to the position of the tropical rain belt, associated with the Inter-Tropical Convergence Zone, and El Niño-Southern Oscillation patterns (Dupont et al. 2010, Srygley et al. 2010). Higher precipitation frequency during the rainy season may create lower ambient temperatures that allow these species to exhibit higher foraging activity during the sampling period, thus indicating low thermal plasticity and/ or critical thermal maximums (García-Robledo et al. 2016, Gonzalez et al. 2020). Increased precipitation following a prolonged dry period may result in increased foraging activity, by increasing availability of floral resources in the tropics, reflected as higher observed bee abundances during the rainy season than the dry season (Maia-Silva et al. 2015, Aleixo et al. 2017). Fluctuations in Ceratinini seasonal abundances may also be due to seasonality in reproduction, as seasonal hibernacula have also been observed (Michener 1985, Santos et al. 2019). However, shifting patterns of precipitation could alter the timing and duration of flowering, and more frequent droughts during the onset of the rainy season could shorten the duration of the peak flowering season as well as the numbers of flowers produced per individual plant, reducing foraging activity and, ultimately, the average life span of pollinator species (Van Schaik et al. 1993, Aleixo et al. 2014).

Since tropical insects are generally regarded as thermal specialists (Dixon et al. 2009), the temperature range that supports physiological function is narrow (Janzen

1967, Sunday et al. 2014). Observed species' preferences for the lower elevation zone (800-900 m) in my study system may be explained by the mid-domain effect, which may exhibit a relatively higher amount of pressure on the distribution of species more likely to be constrained by geographic boundaries (Jetz and Rahbek 2002, Colwell et al. 2004). For example, the distribution of larger-ranged species, as found in geometrid moths along an elevational gradient in Costa Rica (Brehm et al. 2007), were more likely to be constrained by geographic boundaries than smaller-ranged species, whose distributions have been shown to be driven by the effects of environmental or historical factors (Rehan et al. 2010, Carneiro et al. 2014). If tropical bees follow this trend, then my results indicate that the mid-domain effect would exhibit higher pressure on the distribution of larger-ranged species, such as *M. fasciata* and *M. fallax* (Araujo et al. 2004), as these species are more likely to be constrained by the mid-domain effect than smaller-ranged species, such as *C. chloris* and *C. zexmeniae*, and are, therefore, at a higher risk of local extirpation (Sunday et al. 2014). However, studies examining the distribution of leaf-litter ants in Costa Rica found that temperature was the leading predictor for determining species richness and ant worker density, and the observed ranges across the elevational gradient revealed species with broad ranges at lower elevations and species with narrow ranges at mid to upper elevations (Longino and Colwell 2011). Some species, including *M. beechii*, *M. fasciata*, and *N. mellaria*, reflect these distribution patterns, with *M. beechii* exhibiting a narrow range at 1000-1100 m, and *M. fasciata* and *N. mellaria* exhibiting a broad elevation range from 0-900 m. However, other species, such as *C. chloris* and *T. perangulata*, exhibit narrow ranges at lower elevations. Moreover, my findings support the hypothesis that there may be a threshold beyond which temperature and resource abundance begin to limit montane insect abundance at higher elevations, as is thought to be a major limiting factor for invertebrate pollinators in the tropics (Colwell et al. 2008).

In addition to the threats many species face in response to warming temperatures (Hegland et al. 2009), climate-related extirpation has been documented at significantly higher rates for tropical species than temperate species, and prominent extirpation risk is expected for montane species as they shift upward in elevation, limiting accessible habitat and floral resources (Chen et al. 2009b, Beck et al. 2016). Mutualistic partners face additional threats in response to climate change, as species are not likely to respond to warming temperatures similarly (Bartomeus et al. 2011); Plant and pollinator species may not be capable of rapidly shifting to a new climate envelope, resulting in spatial mismatches among mutualistic partners, or extinction (Colwell et al. 2004, Burkle et al. 2013), and phenological shifts, such as timing of emergence or flowering, may be altered by warming temperatures resulting in temporal mismatches among mutualistic partners (Kearns et al. 1998, Hoiss et al. 2015, Pacheco Filho et al. 2015).

Elevation specialization may be driven by many individual or combined variables, including temperature and precipitation patterns, species-specific critical thermal maximums, landscape variation and habitat fragmentation, floral resource use, and mutualistic relationships (Deutsch et al. 2008, Colwell et al. 2008, Maia-Silva et al. 2015, Basu et al. 2016). Calculating the critical thermal maximum for each focal species may aid in discerning the role of temperature in patterns of elevation specialization, as well as determining the role that bee morphological traits play in determining species thermal limits (García-Robledo et al. 2016, Gonzalez et al. 2020). Additionally, exploring habitat heterogeneity across the three replicate transects in my study system is necessary in order to further understand how landscape variability plays a role in determining bee community composition and individual species distributions (Raffa et al. 2008, Brosi et al. 2008). Interspecific interference competition may play a part in determining bee species' distributions across an elevation gradient, as individuals compete for available and preferred floral resources in a geographically bounded system (Pinkus-Rendon et al. 2005, Aslan et al. 2013). Additionally, flowering duration, daily and across seasons, is expected to vary in response to increasing temperatures and sporadic rainfall patterns due to climate warming (Opler et al. 1976, Newstrom et al. 1994). In response, bee species may be at an even higher risk for interspecific competition as spatial and temporal phenological mismatches with key mutualistic partners provide an unreliable food resource (Junker et al. 2013, Goulson et al. 2015).

Tropical forests contain 60% of global biodiversity in 6% of the global surface area (Bawa 1990), and 98-99% of the plant species producing the food resource base for tropical wildlife rely on animal pollination (Deutsch et al. 2008), yet few studies have examined the response of bee species to warming temperatures, and most are limited to temperate zones (Chen et al. 2009b). Dry forests make up approximately 42% of tropical vegetation (Murphy and Lugo 1986). The seasonally arid Pacific slopes of the Central American mountains not only harbor the most endangered tropical forest type (Janzen 1988, Stan and Sanchez-Azofeifa 2019), but they also harbor the most at-risk species assemblages under modified climate scenarios primarily due to drastic alterations in precipitation regimes (McCain and Colwell 2011). Despite the biomes importance, Dry forests remains severely understudied compared to humid forests (Stan and Sanchez-Azofeifa 2019). Dry forests contain many endemic species, as well as understudied plant groups and invertebrates (Olesen and Jordano 2002). Consequently, future work should aim to focus on this poorly studied ecosystem and prioritize long-term studies of plant-pollinator relationships and physiological ranges in order to gain a deeper understanding of the driving factors in elevation range specialization. Tropical dry forests are also mostly only found in Northern Costa Rica where there are short mountains. My findings suggest a high degree of elevation specialization in neotropical montane pollinators, however, establishing a connection between elevation specialization and the factors driving that specialization can be difficult due to natural fluctuations in tropical species populations over time (Wolda 1988, Sheldon 2019), and the lack of long term data (Sakai and Kitajima 2019). Nevertheless, more research is necessary to understand large-scale patterns of species diversity, specifically regarding pollinators, as plant-pollinator relationships are an invaluable ecosystem service to biodiversity maintenance and humanity (Klein et al. 2008, Sánchez-Bayo and Wyckhuys 2019).

POLLINATOR COMMUNITY COMPOSITION OF *PERSEA AMERICANA* AND *CUCURBITA PEPO* ON AN ELEVATION GRADIENT: IMPLICATIONS FOR CROP POLLINATION

INTRODUCTION

Plant-pollinator interactions are a critical ecosystem service of great economic and agricultural value; without pollinators, many plants could not set seed and reproduce, and without plants to provide rewards, such as pollen and nectar, many animal populations would decline (Kearns et al. 1998). Plant-pollinator interactions are under increasing threat from anthropogenic sources, including fragmentation of habitat, changes in land use, modern agricultural practices, and invasions of non-native plants and animals (Vanbergen and the Insect Pollinators Initiative 2013). Animal pollination is thought to be a key factor in the diversification of some major groups of plants and animals, as animal-mediated pollination permits for greater genetic variability, diversity, and robustness to extinctions within populations of plants by allowing for out-crossing of genetic material, leading to a greater diversity of resources for animals at higher trophic levels (Johnson 2010, Neuschulz et al. 2016). In tropical communities, 94% of flowering plants are animal pollinated, and 98-99% of the plant species producing resources for wildlife in tropical forests rely on animal-pollination (Bawa 1990, Ollerton et al. 2011, Watts et al. 2016). Pollination services are of particular importance for maintaining biodiversity in tropical areas that are considered to be epicenters of biodiversity (Balvanera et al. 2005, Kennedy et al. 2013), as biotic pollination importance increases with decreasing latitudes (Olesen and Jordano 2002).

The value of the ecosystem services provided by pollinators can be demonstrated by human reliance on animal-pollinated crops; 75% of the world's leading food crops show increased fruit or seed set when exposed to animal-mediated pollination (Kearns et al. 1998). While pollination services are key to the pollination of many crops and wild plants, crop pollination is commonly specified as an endangered ecosystem service (Corbet et al. 1991, Allen-Wardell et al. 1998, Klein et al. 2007). Smallholder agriculture represents 83% of the global agricultural population (> 2 billion people) and many of the agricultural plantations present in the tropics (Garibaldi et al. 2016); many economically important tropical crops such as coffee, habanero peppers, cashews, and West Indian cherry depend on bees for pollination services (Freitas et al. 1999, Macias-Macias et al. 2009, Classen et al. 2014). However, pollination services in tropical montane ecosystems are potentially more sensitive to climate change than lowland or temperate systems as they consist of a higher proportion of functionally specialized interactions (Olesen and Jordano 2002). Plant-pollinator interactions may be particularly sensitive to climate change-associated shifts in temperature and precipitation regimes (Theobald et al. 2016), causing phenological mismatches among mutualistic partners, and increasing the risk of widowhood, local extirpation of plants and pollinators, and subsequent cascading effects on biodiversity (Bartomeus et al. 2011, Aslan et al. 2013, Sánchez-Bayo and Wyckhuys 2019). While much work has focused on quantifying pollination services across land use gradients, research aimed at understanding if pollination services differ along elevational gradients is scarce, despite the important implications for how warming temperatures might impact pollination.

Bees are considered to be the most highly effective pollinators due to the presence of specialized hair-like structures used to collect pollen, as well as their necessity for pollen, nectar, and essential oils for brood, and specialized foraging behaviors (James and Pitts-Singer 2008). Globally, bee species face multiple threats including climate change, deforestation, pesticides, agricultural intensification and invasive species, and Neotropical bee populations are as threatened as global bee populations (Freitas et al. 2009). The Pantropical bee tribe Meliponini consists of species that are social, stingless bees that produce honey and whose native range is limited to the tropics, subtropics, and the temperate areas of the southern hemisphere (Kaluza et al. 2018). Meliponini have been found to be important pollinators in the tropics, as previous studies have found species in this tribe to be the primary pollinators of coffee and contribute to the pollination of >60 other tropical crops (Ricketts et al. 2004, Brosi 2009, Peters et al. 2012, Campbell et al. 2018). Meliponini are also generally considered to be polylectic, generalist foragers, rather than specializing on specific plant species or traits (Eltz et al. 2001). Other neotropical bee species that have been found to be important crop pollinators belong to the tribes Euglossini, Eucerini, Ceratinini, and the introduced bee species *Apis mellifera* (Jha and Vandermeer 2009, Briggs et al. 2013, Delgado-Carrillo et al. 2018).

In tropical agroforests, two insect-pollinated plantation crops, squash and avocado, represent two different breeding systems and are predicted to receive a diverse suite of pollinators and bee functional groups. Avocado, *Persea americana* Mill. (Lauraceae), is native to Central America and has a protogynous dichogamy reproductive strategy that ensures cross-pollination (Mcgregor 1976, Ish-Am and

Eisikowitch 1993). Stingless bees from the tribe Meliponini have been found to be effective pollinators of avocado, within Meliponini native ranges (Can-Alonzo et al. 2005, Perez-Balam et al. 2012), however, *Apis mellifera* have been found to be the primary, yet unreliable, pollinators of avocado outside of avocado native ranges (Ish-Am et al. 1999). Squash, *Cucurbita* spp. (Cucurbitaceae), is a globally recognized important food crop and is commonly utilized in pollination service studies due to the obligate outcrossing nature of the breeding system (Vidal et al. 2010). Seed set in *Cucurbita* spp. is directly correlated to measures of pollination success due to pollen limitations on seed production (Mcgregor 1976, Kishan et al. 2017). The primary pollinator species of *Cucurbita* spp. have been shown to vary between the wet and dry seasons in tropical dry forests; genera belonging to the tribe Eucerini, *Peponapis* and *Xenoglossa,* have been found to be the most frequent and effective pollinator visitors during tropical dry forest wet seasons, whereas *Apis mellifera* were found to be the most frequent pollinating visitor during the tropical dry forest dry season (Tepedino 1981, Delgado-Carrillo et al. 2018).

Declines in global pollinator abundances has resulted in a reduction in crop yields and crop pollination services (Millenium Ecosystem Assessment 2005). The paucity of scientific studies aimed at understanding how warming temperatures and changing patterns of precipitation may impact this essential and endangered ecosystem service is remarkable given the significant value of crop pollination for global food security. Some studies have found that native pollinator species are more effective in fruit and seed production than introduced species (Garibaldi et al. 2013, Gibbs et al. 2016). Honey bees continue to be critical pollinator species for many agroecosystems

(Perez-Balam et al. 2012), yet a global decline in honeybee populations and the nature of the honeybee's pollination method may make this species unreliable for sustaining or increasing crop yields (Klein et al. 2003, Ricketts et al. 2004). Here, I focus on quantifying pollinator assemblages to *Cucurbita* spp. and *Persea americana* within their native ranges, and along an elevational gradient in the tropics, in an effort to understand whether tropical pollination services differ along elevational gradients. My main objectives were to (i) quantify bee community composition at both crop species along an elevational gradient, (ii) calculate bee community similarity, comparing elevations spanning two agriculturally important tropical life zones, the lower zone of Tropical Dry Forest, and an upper zone of Tropical Premontane Forest. For *Cucurbita pepo*, bee visitation rates were determined across an elevational gradient during the wet (May-November) and dry (December- April) seasons of 2019. For *Persea americana,* bee visitation rates across the elevation gradient were quantified during its flowering period in December of 2018 and 2019.

METHODS

Study Site

Fifteen smallholder farms and residential properties were located on an elevation gradient spanning 700-1150 m above sea-level in the San Luis Valley of Monteverde, Puntarenas, Costa Rica (10˚16’ N, 84˚48-49’ W; **Figure 2-1**). The San Luis Valley is located on the Pacific slope of the Tilarán mountain range from approximately 500 to 1200 m above sea-level. The study area is comprised of tropical dry forest, with low humidity during the dry season (20-60% day time average humidity; Janzen 1988), and pre-montane forest with consistently perhumid conditions (Freiberg 1997), and daily temperatures averaging ~19 ˚C (Harvey 2000). The rainy season lasts from May to November, with mean precipitation of 200-600 mm per month.

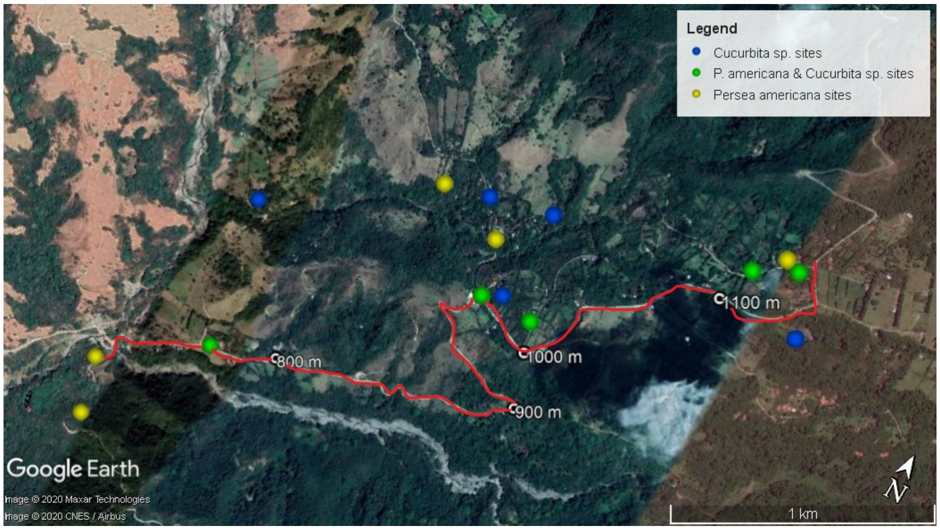


Figure 2-1. Sites selected for *P. americana* and *C. pepo* sampling across an elevational gradient located in San Luis de Monteverde, Costa Rica. Yellow markers represent *P. americana* sites, blue markers represent *C. pepo* sites, and green markers represent sites where both focal crop species were sampled. Meters above sea-level are shown for each 100-meter transition across the transect. Obtained from Google Maps.

The dry season typically takes place from December to April with a rainfall average of 50-150 mm rainfall monthly (Baker 2007, Peters et al. 2010). The region consists primarily of pastures, sugar cane and coffee plantations, and other agricultural plantations subdivided by patches of old primary forest (Peters et al. 2010). This site is protected under conservation easements that preclude forest-clearing activities.

Observations were grouped into 100 m elevation bands and assigned elevation categories as follows: Observations conducted between 651-750 m were categorized in the 700 m elevation band; observations conducted between 751-850 m were categorized in the 800 m elevation band; observations between 851-950 m were categorized in the 900 m elevation band; observations between 951-1050 m were categorized in the 1000 m elevation band; and, observations conducted between 1051-1150 m were categorized into the 1100 m band. Two farm sites occurred in the 700 m elevation band, two sites in the 800 m band, three in the 900 m band, four in the 1000 m band, and four sites in the 1100 m elevation band. Sites containing avocado trees were located across the elevation gradient, while squash seeds were planted directly in the ground at sites selected for utilization in squash sampling. Ten of the fifteen farm sites were utilized for *P. americana* sampling as well as for *C. pepo*. sampling. The San Luis Valley encompasses small forest patches, windbreaks, cattle pastures, and sugarcane and coffee plantations. In order to incorporate the diverse nature of the landscape in the study area, the farms selected for this study varied between coffee agroforests and smaller residential farms.

Focal Crop Species

Avocado, *Persea americana*, is a tropical evergreen tree native to Mexico, Central and South America, however, it is widely and commercially cultivated in tropical and Mediterranean climates globally (Chen et al. 2009a). Worldwide, the avocado industry produces more than 4 million tons of avocados for consumption yearly, equivalent to approximately nine billion USD (González-Estudillo et al. 2017). Avocado trees are commonly 15-30 feet tall, but can grow up to 60 ft tall, and consist of inflorescences of inconspicuous light green blossoms (**Figure 2-2**). Avocado flowering behavior expresses a diurnally synchronous protogynous dichogamy; each flower opens twice, first as a female, then as a male during the second opening, rendering each avocado flower structurally and functionally bisexual (Bender 2002). The two complementary flowering stages occur simultaneously on separate individuals and, under proper conditions, will flower at opposite stages in order to ensure cross-pollination (Bergh 1973). However, as temperatures are variable, the daily openings of functionally male and female flowers may become delayed and irregular, such that a single tree may have female and male flowers open at the same time, allowing self-pollination to occur (Silva et al. 2002). Studies of avocado pollination in Central America revealed that bees from the tribe Meliponini show a significant preference for avocado blooms, while honeybees exhibit activity on avocado blooms but readily forgo avocado for competing or more rewarding flowers (Ish-Am and Eisikowitch 1993, Ish-Am et al. 1999).

Figure 2-2. Single *Persea americana* flower during stage two, the functionally male stage. *Source*: Kristina Victoreen, Earthwatch Institute volunteer, December 2019.

Cucurbita, one of approximately 90 genera belonging to Cucurbitaceae, consists of species commonly known as squash, gourds, pumpkins, and zucchini, and is native to Mesoamerica (Wilson 1990). *Cucurbita* species are annual vine or bush crops that are monecious, thus requiring biotic pollination for fruit set (Hurd et al. 1971, Willis and Kevan 1995). Squash varieties are widely grown for commercial use and are utilized for both human consumption and livestock fodder (Isaacs et al. 2017). *Cucurbita pepo* produces large, showy yellow-orange flowers with male flowers producing an abundance of pollen and nectar, and female flowers producing copious amounts of nectar (Mcgregor 1976, Tepedino 1981). Squash flowers have a brief flowering period; flowers open before or by sunrise and close by the afternoon (Hurd et al. 1971, Willis and Kevan 1995). Studies of *Cucurbita* pollination have revealed that *Peponapis* and *Xenoglossa* genera of the tribe Eucerini are obligate pollinators (Tepedino 1981), however, honey bees and stingless bees are occasionally important pollinators of squash outside of Eucerini species' ranges and when weather and climatic conditions do not favor Eucerini foraging activity (Michelbacher et al. 1964, Heard 1999).

Pollinator Sampling

Pollinator sampling was conducted during the months of June-July and December of 2018-2019 from 0800 to 1400 h daily in order to quantify the pollinator assemblages for *P. americana* and *C. pepo* during their respective flowering seasons and explore the relationship between the pollinator assemblages and altitude across an elevation gradient. GPS units were utilized in order to obtain the longitude, latitude, and altitude of each focal crop individual at each site during the sampling period. Ten *P. americana* individuals were selected based on accessibility and evenness across the sampled elevations.

Active collection methods were employed as this is the most effective way to target the entire bee community. Any bees present during the sampling period were captured using hand nets and jars and preserved using 70% ethanol. Floral abundance for squash was counted, and avocado was estimated by counting the flowers on 3 to 4 branches and multiplying by the number of branches. Samples were exported to Eastern Kentucky University for identification and processing. All bee specimens were keyed out to species or genus using identification keys in Michener (2007), Mawdsley (2017), Jarau and Barth (2008), and Aguiar and Melo (2011).

Statistical Analysis

To determine the adequacy of sampling effort, species accumulation curves for each focal crop species were formulated using the *accumcomp* function in the BiodiversityR package of R version 2.11-3 (Kindt and Coe 2005). Species names were abbreviated for clarity (**Appendix A**).

To compare bee community composition of each elevation band, the degree of similarity of the community composition among the elevation categories for each focal crop species was analyzed using the *'SimilarityMult'* function in the 'Spade-R' package of R version 0.1.1 (Chao et al. 2016), using the Morisita index to conduct multi-stage community comparisons. This approach is the most robust method for comparing community overlap as it allows for concurrent analysis of several samples and among communities with a species overlap greater than two (Chao et al. 2008). Communities were considered to be statistically different if similarity values were less than 0.19 (Rodríguez-Castañeda et al. 2010).

RESULTS

A total of 47 samples were collected from *Persea americana* in December of 2018 and 2019, resulting in 145 individuals representing 21 species (**Figure 2-3**). Pollinator species observed visiting *P. americana* belong to two bee families, Apidae and Halictidae, and included the tribes Apini, Augochlorini, Ceratinini, Chilicolini, Emphorini, Halictini, and Meliponini. The species accumulation curve for *P. americana* did not appear to approach an asymptote, indicating the need for further sampling in order to accurately assess the bee community composition of *P. americana* (**Figure 2-4**).

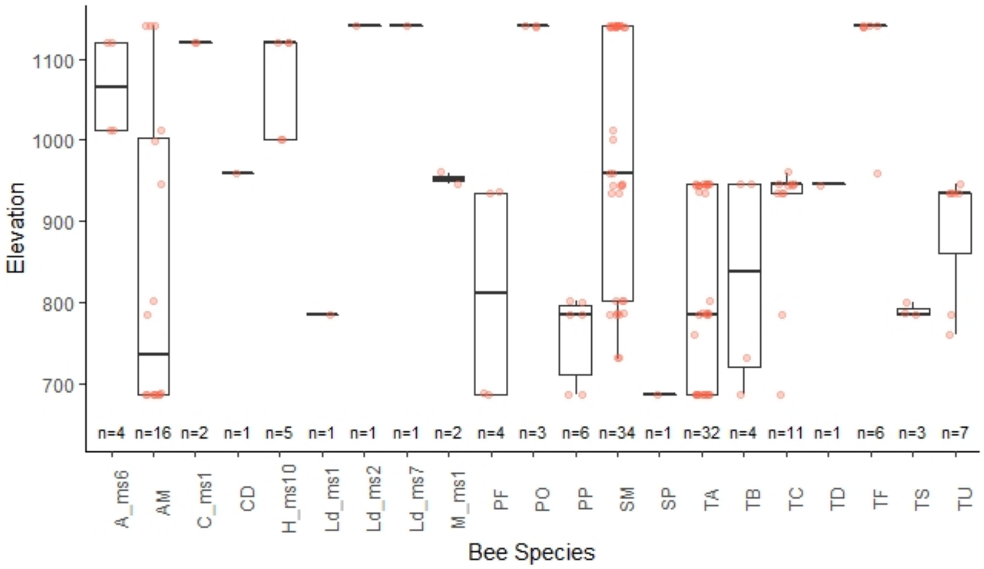


Figure 2-3. Abundance of species collected during thirty-minute timed flower observations at *Persea americana* across the elevation gradient. Each red dot corresponds to on individual. Observations were conducted during December of 2018 and 2019 in the San Luis Valley, CR.

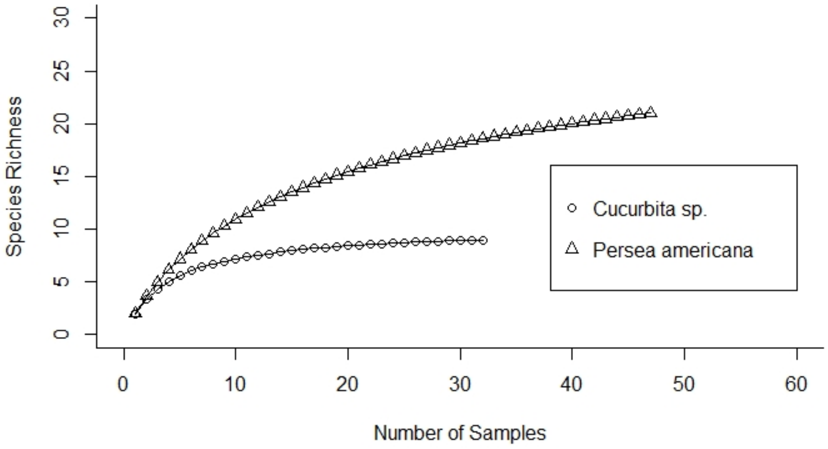


Figure 2-4. Species accumulation curves for timed samples conducted at flowers of *Persea americana* and *Cucurbita pepo*, 2018-2019.

A total of 32 samples were collected from *Cucurbita pepo* during the wet and dry seasons of 2019, resulting in 223 individuals representing nine species (**Figure 2-5**). Pollinator species collected from *C. pepo* flowers were from two bee families, Apidae and Halictidae, and included the tribes Ceratinini, Eucerini, Halictini, and Meliponini. The species accumulation curve for *C. pepo* indicated that My sampling effort was adequate.

Multiple stage similarity comparisons were conducted on all bee observations collected from each focal crop species. The similarity among observations across all sampled elevations were compared in order to determine if bee communities significantly differed (similarity values ≤ 0.19) in response to elevation. The bee community visiting *P. americana* flowers differed among the elevation categories (**Table 2-1**).

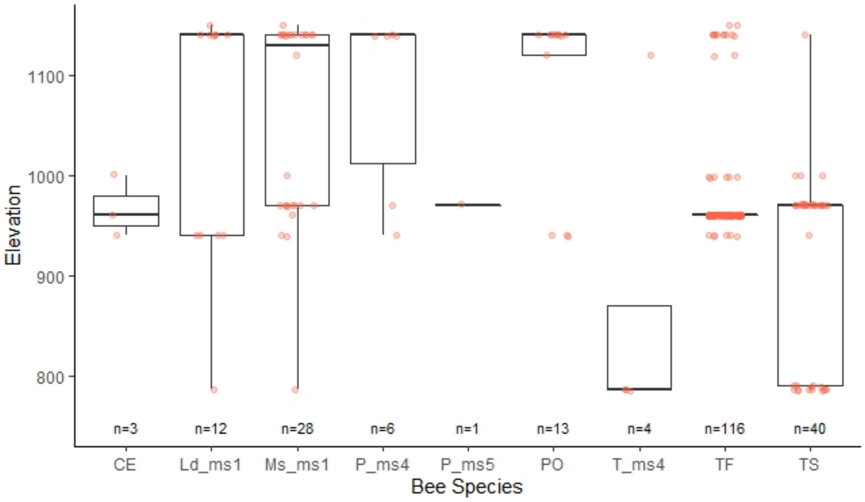


Figure 2-5. Abundance of species collected during thirty-minute timed flower observations at *Cucurbita pepo* across the elevation gradient. Red dots indicate individual observations. Observations were conducted during the wet and dry seasons of 2019.

Table 2-1. Community similarity matrix observed at *Persea americana*, December 2018 and 2019.

Elevation Category	700 m	800 m	900 m	1000 m	1100 m
700 m	1.000	0.809	0.804	0.393	0.255
800 m	---	1.000	0.883	0.691	0.656
900 m	---	---	1.000	0.461	0.346
1000 m	---	---	---	1.000	1.000
1100 m	---	---	---	---	1.000

Bee communities at 1000 m and 1100 m elevation categories exhibit the highest degree of similarity for *P. americana* (mean Morisita similarity value *c.* 1.000), while bee communities at 700 m and 1100 m elevation categories exhibit the highest degree of dissimilarity (mean Morisita similarity value *c.* 0.255). The community present within the 1100 m elevation category exhibited the highest degree of dissimilarity with all other elevation categories, except for the community present at the 1000 m elevation category. Similarity comparisons for *C. pepo* demonstrated high dissimilarity values and high turnover among the elevation categories (**Table 2-2**). Bee communities at 900 m and 1100 m elevation categories exhibit the highest degree of similarity for *C. pepo* (mean Morisita similarity value *c.* = 1.000), while the bee communities at 800 m and 900 m, as well as 800 m and 1100 m elevation, were the most dissimilar (mean Morisita similarity value *c.* = 0.164, mean Morisita similarity value *c.* = 0.105) respectively.

Table 2-2. Community similarity matrix observed at *Cucurbita pepo*, 2019.

Elevation Category	800 m	900 m	1000 m	1100 m
800 m	1.000	0.164	0.248	0.105
900 m	---	1.000	0.726	1.000
1000 m	---	---	1.000	0.582
1100 m	---	---	---	1.000

DISCUSSION

Pollination of crops by native pollinator species is an invaluable ecosystem service (Ashworth et al. 2009) in the Neotropics, where 94% of plant species rely on animal pollination, and many rural populations rely heavily on subsistence farming (Cole 2010, Ollerton et al. 2011). My findings emphasize the importance of native pollinators in crop production, as an introduced bee species, *Apis mellifera*, was found in only ~11% of *P. americana* observations and in no observations of *C. pepo*. Pollination by native bee species has been shown to be as effective as pollination by managed bees (Freitas and Paxton 1998, Heard 1999), however, the maintenance of native bee species is dependent on the preservation of suitable habitat within agricultural landscapes (Ricketts et al. 2004), as well as the conservation of plant-pollinator mutualisms (Tylianakis et al. 2010). The Main road transect was established in 2012 as part of a larger study investigating pollinator abundance and species richness patterns across elevational gradients, and has since found ~180 bee species to be present in the study area, however, this number may underrepresent the actual number of bee species present as sampling efforts have been, at least in part, focused on passive sampling techniques that may under sample the bee community on farms and residential properties (Roulston et al. 2007, Prendergast et al. 2020).

The pollinator assemblage of *P. americana* in my study system consisted of 21 species, comprising approximately 12% of the total observed species richness in the study area. Approximately 77% of the individuals observed visiting *P. americana* belong to the tribe Meliponini, while the other 23% of pollinator observations consisted of the tribes Apini, Augochlorini, Ceratinini, Chilicolini, Emphorini, and Halictini.

Similarly, of the nine species and 223 individuals observed visiting *C. pepo*, comprising approximately 5% of the total observed species pool, ~76% of those individuals belong to the tribe Meliponini, while 24% of the observations consisted of species belonging to the tribes Ceratinini, Halictini, and Eucerini. Eucerini tribe genera *Xenoglossa* and *Peponapis* have a close ecological relationship with and are specialist foragers for *Cucurbita* sp., however, species belonging to the tribe Eucerini make up only ~5% of all individuals observed at *C. pepo*, indicating the need for further sampling in order to determine if Eucerini species are rare in the sampling area and the extent to which Meliponini species provide effective pollination services. Furthermore, Meliponini are thought to be important pollinators for avocado as they have similar historical and contemporary ranges and have been documented as frequent avocado visitors (Can-Alonzo et al. 2005, Slaa et al. 2006). My findings suggest that Meliponini species may be important pollinators for *P. americana* and *C. pepo* in the study area, and conservation efforts should work to ensure the preservation of this bee tribe.

The relatively infrequent observation of Eucerini species visiting *C. pepo* in my study may be explained by interspecific interference competition due to overlap in resource use, if species belonging to this tribe are truly rare in the study area, as rare species can be displaced by common species due to overlap in resource use (Steffan-Dewenter and Tscharntke 2000). Species in the tribe Eucerini comprise ~1% of all individual bees observed in the study area, although sampling efforts have been primarily focused on treelets, shrubs, and herbaceous growth, as well as passive sampling, thus sufficient sampling of preferred plant species belonging to Cucurbitaceae may discern this bee tribe's rarity in the study area.

After ~16 hours of *Cucurbita pepo* observations and ~23.5 hours of *Persea americana* observations, species accumulation curves indicate that further sampling may be needed for *P. americana* in order to accurately determine the pollinator assemblages visiting each focal crop species and the variation in assemblages across the elevation gradient. While sampling efforts remain too low to be certain of the degree to which elevation specialization in bee species may affect the variation in the pollinator assemblages occurring at *P. americana*, community similarity analyses for each focal crop species revealed relatively high variation in the similarity of the bee communities visiting each crop species across the elevation gradient. For *P. americana*, many community comparisons resulted in a degree of similarity greater than 50%, while communities at the highest sampled elevation categories exhibited low similarity values with lower elevations; the bee community visiting *P. americana* at the 800 m elevation category exhibited a low degree of similarity with the communities at the 1000 m and 1100 m elevation categories. Similarly, the pollinator assemblages observed at *C. pepo* exhibit a significant degree of dissimilarity, particularly among communities observed at 800 and 900 m, and at 800 and 1100 m, indicating the importance of elevation in determining the pollinator assemblages occurring at each crop species, as well as crop pollination effectiveness (Theobald et al. 2016). Elevation specialization by bees may indicate sensitivity to temperature or precipitation patterns, competition avoidance, species-specific thermal maximums, floral resource availability, or habitat preference (Deutsch et al. 2008, Colwell et al. 2008, Basu et al. 2016).

Pollinator dependent food crops are more profitable than non-pollinator dependent crops (Ashworth et al. 2009), and food crop pollination in rural tropical agroforests is highly dependent on the native pollinator community (Klein et al. 2008). Presence of natural habitat is also important to ensuring crop pollination effectiveness, as increased variation in pollinator success and fruit set has been observed for crops located in intensive agricultural areas or isolated from natural habitat (Eeraerts et al. 2019). Understanding the pollinator assemblages occurring at *P. americana* and *C. pepo*, and how pollinator distributions may be influenced by elevation and climatic variables, is fundamental to supporting crop production in tropical montane regions (Macias-Macias et al. 2009). My results suggest that species in the tribe Meliponini may be key pollinators for both *P. americana* and *C. pepo*, as both focal crop species received approximately 75% of pollinator visits from species in this tribe. Obtaining fruit set or weight, as well as seed set, for my crop species, may help to elucidate pollination effectiveness (Sheffield 2014), as well as employing other pollinator sampling methods, including pollen grain assessments and pollinator exclusion methods (Eltz et al. 2001, Magrach et al. 2018). Many variables may be important in determining the pollinator visitors to *P. americana* and *C. pepo*, however, understanding the factors that drive species distribution patterns across elevation gradients, as well as promoting the management of natural areas and sustainable farming, are critical to conserving plant-pollinator relationships (Colwell et al. 2008, Gonthier et al. 2014, García-Robledo et al. 2016).

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APPENDICES

APPENDIX A:

List of abbreviations used in figures in chapter 2 in place of species complete scientific name.

List of abbreviations used in figures in chapter 2 in place of species complete scientific name.

Species	Abbreviation
Apis mellifera	AM
Augochloropsis morphospecies 6	A_ms6
Ceratina dimidiata	CD
Ceratina eximia	CE
Chilicola morphospecies 1	C_ms1
Halictini morphospecies 10	H_ms10
Lasioglossum dialictus morphospecies 1	Ld_ms1
Lasioglossum dialictus morphospecies 2	Ld_ms2
Lasioglossum dialictus morphospecies 7	Ld_ms7
Melissodes morphospecies 1	Ms_ms1
Melitoma morphospecies 1	M_ms1
Partamona orizabaensis	PO
Plebeia frontalis	PF
Plebeia pulchra	PP
Peponapis morphospecies 4	P_ms4
Peponapis morphospecies 5	P_ms5
Scaptotrigona mexicana	SM
Scaptotrigona pectoralis	SP
Tetragona dorsalis	TD
Tetragonisca angustula	TA
Thygater morphospecies 4	T_ms4
Trigona corvina	TC
Trigona fulviventris	TF
Trigona fuscipennis	TU
Trigona silvestriana	TS
Trigona buyssoni	TB