



High Foraging Fidelity and Plant-Pollinator Network Dominance of Non-native Honeybees (*Apis mellifera*) in the Ecuadorian Andes

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Abstract

Pollinator foraging fidelity (i.e., consistent and repeated visitation to a particular plant species or area) is poorly understood for most bee species, but is important information for both the conservation of plant and pollinator species and the ecosystem services they provide to humans. We used plant–pollinator surveys and mark–recapture of floral-visiting Hymenoptera to study the foraging fidelity and species interaction network properties of a plant–pollinator community in the tropical Andes of southern Ecuador. After marking 92 bees visiting six plant taxa along four 100-m transects between July 16th and July 31st of 2019, only honeybees were resighted at a recapture rate of 47.7% (41/86). During our surveys, we observed nine bee and two wasp taxa feeding from the flowers of 10 morphospecies of plants, and we found significantly low network nestedness and significantly high network-level specialization. Specialization (d') was also significant for honeybees and bumblebees and for three plant taxa. Overall, our findings indicated that feral, non-native honeybees in this region dominated the local plant–pollinator network, yet this species is acting as a specialist forager at the individual level. Our results suggest that honeybees may be replacing the pollination services of some native bees and wasps in the region, but more research is needed to determine the effectiveness of honeybee pollination for the local plants.

Keywords *Bombus* · Foraging behavior · Fragmentation · Halictids · Hymenoptera · Mark–recapture

Bees and wasps (Hymenoptera: Apoidea) are important pollinators worldwide, both for native flowering plants as well as crops (Levin 1983; Klein et al. 2007). Understanding pollinator foraging fidelity (i.e., returns to a particular plant species or area) is crucial for conserving these species and the ecosystem services they provide in the face of anthropogenic impacts to their habitats. Mark–recapture methods permit studying the short-term foraging fidelity of these species (Ogilvie and Thomson 2016; Cecala and Wilson Rankin 2020). Surprisingly, despite the nearly worldwide ubiquity and pollination importance of the western honeybee (*Apis*

mellifera Linnaeus), we could find no mark–recapture studies of the foraging fidelity of this species. Although a few mark–recapture studies have examined solitary bee species [see references in Cecala and Wilson Rankin 2020], most have investigated bumblebees [*Bombus* spp.; Bronstein et al. 2017; Mola and Williams 2019].

Western honeybees have been introduced by humans to ecosystems worldwide for crop pollination and honey production, and they have thrived and spread outside of their native range, in part due to their generalist foraging habits, large colonies, and highly social behavior (Waser and Ollerton 2006; Valido et al. 2019). Conversely, native bees are usually solitary and more specialized foragers (Waser and Ollerton 2006). Competition between introduced honeybees and native pollinators can hinder growth, survival, and reproduction of native species (Paini 2004) and negatively affect local plant–pollinator networks (Schaffer et al. 1983; Kato et al. 1999; Valido et al. 2019).

Our objectives were to use plant–pollinator surveys and mark–recapture of floral-visiting Hymenoptera to study the foraging fidelity and species interaction network properties of a plant–pollinator community in the tropical Andes of

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southern Ecuador. Our research was conducted between 2900 and 3100 m asl at Universidad del Azuay's 120 ha El Gullán research station, roughly 52 km south of the city of Cuenca ($3^{\circ}20'17.35''\text{S}$, $79^{\circ}10'16.89''\text{W}$; Fig. 1). This site is characterized by high-elevation montane shrubland vegetation with seasonal rain patterns consisting of a wet period from September to May and a dry season from June to August (Cárdenas et al. 2017). El Gullán and the surrounding landscapes are highly fragmented due to cattle grazing, and are also threatened by climate change (Chacón-Vintimilla 2016). Little is known about the many endemic species that live in this imperiled habitat.

All data was collected between July 16th and July 31st of 2019 between 10:00 and 14:00 when the sun was out and there was little to no wind. To census the plant–pollinator community, we set up four 100-m transects separated by 100 m. We walked each transect and identified any flowering plants within 2 m on either side of the trail. We then counted the number of open flowers on the plants to estimate plant species–specific resource abundance (Maglianesi et al. 2014).

We chose to collect pollinators from the six most common species of flowering plants along the transects, in an effort to maintain consistency for mark–recapture by taking

a sub-sample of the larger floral community [see Williams et al. 2001]. These morphospecies were *Ageratina pseudochilca* (Asteraceae), *Dendrophorbium* sp. (Asteraceae), *Ageratina* sp. (Asteraceae), *Ilex* sp. (Aquafoliaceae), *Gaultheria reticulata* (Ericaceae), and *Miconia* sp. (Melastomataceae). We then censused the pollinator community, focusing on bees and wasps (Hymenoptera: Apoidea) as these are the most abundant at the field site. Two people collected all bees and wasps they observed visiting flowers on each transect using a hand net, for a total of four person-hours per transect. Captured insects were transferred to small plastic vials for later identification, and we recorded on which flower species each was captured. This survey of bees and wasps occurred either well before (at least 6 days) we conducted mark–recapture of pollinators on the same transect, or after mark–recapture was already complete so as not to affect the outcome of our mark–recapture efforts.

To assess patterns of floral fidelity in honeybees and native bees, we used a mark–recapture approach. In order to mark individual bees, we constructed a tool similar to a queen marking cage. For two person-hours twice a day (once in the morning and once in the afternoon) between the hours of 10:00 and 14:00, we walked each 100-m transect and hand-netted all bees observed on the same six plant

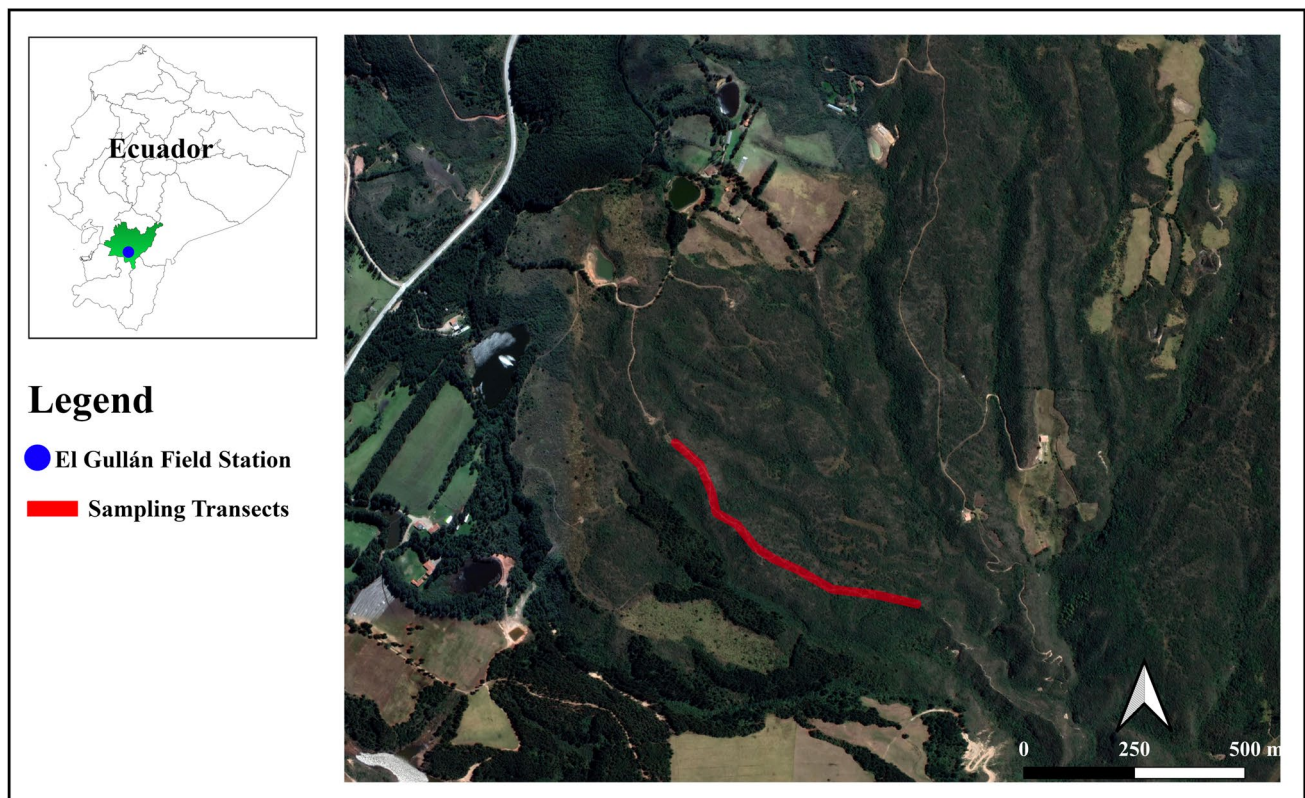


Fig. 1 Study site at the Universidad del Azuay's 120 ha El Gullán research station property and the trail where the sampling transects were located (red line) in the southern Ecuadorian Andes mountains south of Cuenca

species we chose for the survey described above. Captured bees were transferred into the marking cage (Fig. 2a; see Yamamoto et al. 2014; Cecala and Wilson Rankin 2020) and each bee was marked with a dot of color on the thorax with a non-toxic paint marker (Fig. 2b). The color of the paint was specific to the species of flower where the bee was found foraging (Fig. 2c, d). We used six different colors of paint markers to correspond to the six species of flowers, and only marked bees found on those six species. Recapture days took place the day after the pollinators were marked on each transect, following the same time schedule and procedure. When we found a bee that was already marked from the previous day, we marked it with a different color also corresponding to the flower that it was found on so that we did not count it more than once. When we detected unmarked bees on this second day, we noted which flowers they were seen on but did not catch and mark them. The same observer (R. Crafford) participated in all of the marking and recapture transects for consistency.

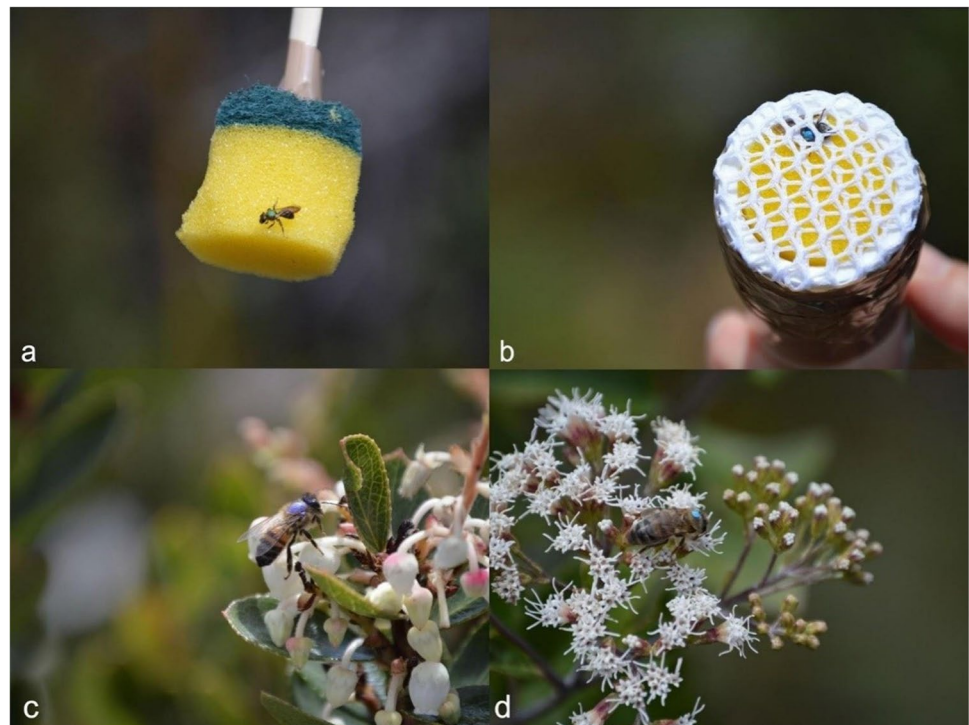
All statistical analyses were conducted in R v. 3.6.3 (R Core Team 2020). To assess which factors influenced probability of recapture, we conducted generalized linear mixed models using ‘glmer’ from the *lme4* package with a binomial error structure (Bates et al. 2013). We included date as a random effect, and flower species and transect as fixed effects. Each observation (i.e., marked bee) was weighted according to the number of individuals marked by bee species, mark color, date, and transect [sensu Cecala and Wilson Rankin 2020]. Transect by flower species interaction was not

included in the final model because it was non-significant ($p = 0.89$) and the final model was significantly improved ($\text{dAIC} > 2$) when the interaction term was removed. For significant factors, we conducted post hoc comparisons using ‘emmeans’ with a Tukey adjustment for multiple comparisons when appropriate (Lenth 2019).

To create the most comprehensive floral networks, we pooled the visitation data of the surveys and mark–capture experiment, which yielded 297 floral visitation observations. To assess the network structure of our plant visitation networks, we calculated network structure metrics using ‘networklevel,’ ‘grouplevel,’ and ‘specieslevel’ in the package *bipartite* (Dorman 2011). To determine if our networks were more structured than random, we developed null networks using the function ‘generate_null_net’ from package *econullnet* (Vaughan et al. 2017) and compared these to observed values, in each case repeating with 1000 iterations. We also used the function ‘test_interactions’ to compare observed interaction strengths (defined as the total number of interactions summed across individual consumers) to those generated by the null model (Vaughan et al. 2017) using a Bonferroni correction for multiple comparisons where appropriate.

To assess patterns in floral abundance, we calculated the relative floral abundance for each plant species per transect by dividing the total number of open flowers per plant species on a transect by the total number of open flowers on that transect. We similarly calculated the relative plant abundance by dividing the number of individual plants of

Fig. 2 Mark–recapture of floral-visiting insects. Images **a** and **b** show the marking cage tool that was used to mark bees that were caught; images **c** and **d** show two marked honeybees on *Gaultheria reticulata* and *Ageratina pseudochilca* with their corresponding paint colors. Photos: R.E.C



a species flowering on a transect by the total number of individual flowering plants on that transect. From the visitation network data, we calculated the visit frequency for each plant species (number of visits to that plant on each transect divided by the total number of visits to all plants on that transect), as well as the relative diversity of visitors to a plant species (number of different floral visitor taxa to a plant species per transect divided by the total number of different floral visitor taxa on that transect). We then used the function ‘cor’ (R Core Team 2020) to assess Pearson correlation coefficients (r).

We marked 92 bees visiting the six plant taxa along the four transects, and a total of 41 bees (44.6%) were recaptured along the transects. Of the four bee taxa marked (*Apis mellifera* and three halictid morphospecies), the most common was the honeybee (86/92). Only honeybees were recaptured and exhibited a high recapture rate of 47.7% (41/86). This resight rate is in line with but higher than the mean results from mark–recapture studies on *Bombus* bees (mean = 38.94%, range = 5.7–80%, SE = 7.83%) and other apids (mean = 31.75%, range = 9.9–57%, SE = 3.84%; see (Cecala and Wilson Rankin 2020, Table S1). Overall, we recaptured bees from *Ageratina pseudochilca*, *Dendrophorbium*, *Gaultheria reticulata*, and *Ilex* sp. The likelihood of recapture was influenced by both transect ($F_{3,115} = 11.81$, $p < 0.0001$) and flower species ($F_{7,115} = 2.35$, $p = 0.028$). Recapture likelihood was higher in transect 2 compared to all other transects (Table 1; $p < 0.05$). In addition, bees were more likely to be recaptured on *Ageratina pseudochilca* than *Gaultheria reticulata* ($Z = 4.03$, $p = 0.0015$). More work is needed to determine if this higher recapture rate is due to plant species factors such as the number of flowers, quantity and quality of nectar, time and duration of blooming, and attractiveness of flowers.

Seventy-four bees and wasps from five different families were collected from the floral visitor survey: Apidae, Colletidae, Halictidae, Crabronidae, and Ichneumonidae (Fig S1). In total, we observed nine bee and two wasp taxa feeding from the flowers of 10 morphospecies of plants (Fig. 3). This total network exhibited 1.38 mean links per species and a connectance of 0.26, yet neither of these metrics differed from null models (Table S2). Interestingly, there was significantly low network-level nestedness (NODF = 45.95; $SES = -3.37$; $p < 0.05$) and high specialization ($H2' = 0.397$;

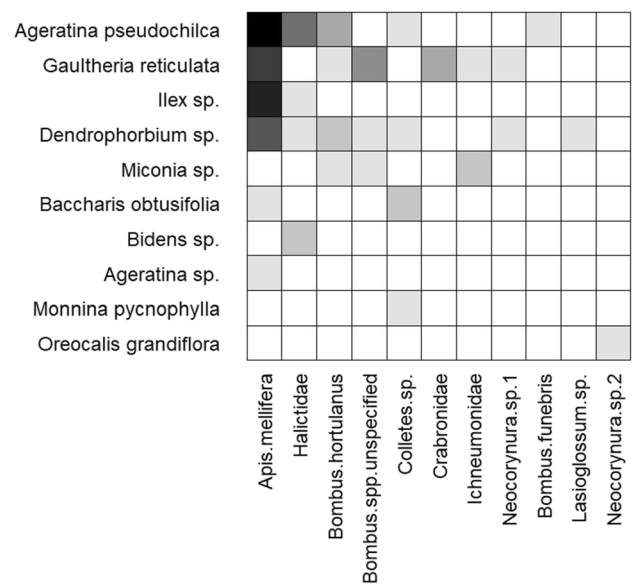


Fig. 3 Plant–pollinator network describing visitation of pollinators (columns) to different plant taxa (rows). The color indicates the relative number of times a visitor was collected off of that species, ranging from white (never observed) to black (most commonly observed). *Bombus* spp. unspecified are bumblebees that we observed foraging but were not collected for species ID

$SES = 7.31$; $p < 0.0001$). Low nestedness is thought destabilize mutualistic networks, and thus indicate communities that may be most sensitive to species loss (Bascompte et al. 2003). Previous studies have also found that disturbed networks exhibit lower nestedness and higher specialization than undisturbed networks (Vanbergen et al. 2017). The high level of habitat fragmentation and abundance of non-native honeybees at our site may explain these results, although longer term studies and comparison control sites without honeybees are needed.

Specialization was also significant at the taxonomic level, where honeybees and bumblebees both exhibited higher d' (specializing on one or a few plants) than null networks (Table S3). While these species are known as super generalists (Goulson 2003), few studies have examined the foraging specialization of individual bees, which are likely to be short-term specialists on plant species that provide reliable resources in order to minimize searching and handling times (Heinrich 1976; Russell et al. 2017).

Table 1 Significant species interactions compared to null network models after Bonferroni p -value correction. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Consumer	Resource	Observed	Null	Test	SES	p adjusted
<i>Bombus</i> (unspecified)	<i>Gaultheria reticulata</i>	4	0.89	Stronger	3.58	0.0375*
<i>Colletes</i> sp.	<i>Baccharis obtusifolia</i>	2	0.10	Stronger	5.783	<0.0001***
Halictidae	<i>Bidens</i> sp.	2	0.18	Stronger	4.335	0.0016**
Ichneumonidae	<i>Miconia</i> sp.	2	0.18	Stronger	4.617	0.0004**
<i>Neocorynura</i> sp.2	<i>Oreocalis grandiflora</i>	1	0.018	Stronger	7.383	<0.0001***

Colletes bees and ichneumonid wasps also were behaving more as specialists than expected based on null networks (Table S3); however, they were much less abundant at our field sites than honeybees and Halictid bees, yet of similar abundance to all other floral visitor species. On the plant side, *A. pseudochilca*, *G. reticulata*, and *Miconia* all had higher levels of d' specialization (fewer different insect visitors) than expected in null networks (Table S3), the first two driven by honeybees and *Miconia* by ichneumonids. In addition, we observed that some interaction strengths in the network were significantly higher than expected. *Colletes* sp. interacted more strongly with *B. obtusifolia* than expected (Table 1; $p < 0.0001$). Bumblebees exhibited significantly stronger interactions with *G. reticulata* ($p = 0.0375$). Halictid bees had stronger than expected interactions with *Bidens* ($p = 0.027$), while ichneumonid wasps strongly interacted with *Miconia* (Table 1; $p < 0.01$).

Visitation frequency by pollinators to a flower was positively correlated with both the relative abundance of a flowering species (Fig S2; $r = 0.75$, $p = 0.0005$) and the relative abundance of the plant ($r = 0.56$, $p = 0.02$). As expected, there was a positive correlation between the relative abundance of flowers and plants (Fig S2: $r = 0.66$, $p = 0.002$). Focusing on the floral visitors, the relative abundance of floral visitors in a transect was negatively correlated with the diversity of the floral visitors present ($r = -0.37$, $p = 0.028$). Unsurprisingly, this was largely driven by a strong positive correlation between the presence of honeybees and floral visitor relative abundance ($r = 0.735$, $p < 0.0001$).

Our findings indicate that feral non-native honeybees in this region have dominated the local plant–pollinator networks. Here, honeybees appear to be specialists at the individual level over short temporal and spatial scales, although as a species *A. mellifera* is known to be a generalist, able to adapt to seasonal variation in floral landscapes (Waser 1986; Amaya Márquez 2009). Halictid bees are also broadly recognized as floral generalists, but their fidelity at the individual level and over small scales is largely uninvestigated (Cecala and Wilson Rankin 2020). Our results contribute insights into the plant–pollinator interactions in this diminishing and fragmented high Andean habitat, and provide a better understanding of the impact and role that honeybees have in habitats where they are introduced. Future studies could compare native plant–pollinator networks in this region that have a low abundance of honeybees to those with a high abundance of honeybees to gain more insight into their effects on the overall community.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13744-022-00967-6>.

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Author contribution All authors contributed to the study conception and design. Rachel E. Crafford collected the data. Erin E. Wilson Rankin analyzed the data and edited the manuscript. Jessie L. Knowlton wrote the first draft of the manuscript. Boris A. Tinoco and Pablo S. Padrón provided taxonomic identifications and manuscript editing. All authors read and approved the final manuscript.

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Data availability Upon request from corresponding author.

Declarations

Ethics approval All authors declare that any authorization needed for collecting biological specimens on environmental protected areas has been obtained from government authorities of the countries where collecting has taken place.

Conflict of interest The authors declare no competing interests.

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