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The pollen foraging ecology of honey bees (Apis mellifera) in a fragmented environment

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The pollen foraging ecology of honey bees (*Apis mellifera*) in a fragmented environment

A Thesis submitted in partial satisfaction of the requirements for the degree of

Master of Science

in

Biology

by

Brian Park

Committee in charge:

Professor James Nieh, Chair
Professor David Holway
Professor Joshua Kohn

2012
The Thesis of Brian Park is approved and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego

2012
DEDICATION

To my family for patiently supporting me in all of my endeavors.

To all of my friends who bring joy to my life.
“What we can measure is by definition uninteresting and what we are interested in is by definition unmeasurable.”

Richard Lewontin, 1974
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ABSTRACT OF THE THESIS

The pollen foraging ecology of honey bees (Apis mellifera) in a fragmented environment

by

Brian Park

Master of Science in Biology

University of California, San Diego, 2012

Professor James C. Nieh, Chair

Honey bees recruit foragers to rich food sources through the waggle dance. The waggle dance has been used extensively to study the foraging ecology of honey bees in various habitats. We decoded waggle dances and used DNA barcoding of bee-collected pollen to characterize the foraging ecology of honey bees (Apis mellifera L.) over 17 months around La Jolla, California, a heavily fragmented environment containing urban, semi-urban, and patches of native scrub habitats. We divided the year into three distinct seasons (dormant, growth, and dry) based on natural patterns of warming and rainfall to understand how honey bee foraging varies over ecologically relevant temporal scales in a
fragmented environment. We detected a significant effect of season on foraging distances. We also found that colonies focused their foraging efforts on few patches during the dormant season and performed increasingly wider searches for pollen with changes in season. Lastly, we detected significant seasonal turnover in the proportion of pollen loads with native or non-native pollen. Bees focused their pollen foraging on native species during the dormant season; both native and non-native species during the growth season; and, non-native species during the dry season. Our results show that honey bees are capable of adjusting their foraging behavior with season to exploit common, abundant native and non-native flowers, illustrating the remarkable adaptability of honey bees in fragmented habitats. Furthermore, our study indicates that honey bees may serve as pollinators of common native plants in light of declines in native pollinators bought on by habitat fragmentation.
Introduction

Upon discovering a rich patch of flowers, a honey bee forager will return to her nest and can advertise this food source by waggle dancing, excitedly moving around the comb in looping figure-eight motions, punctuated by a central waggle-phase. Honey bees only perform this waggle dance to communicate the location of new, highly profitable resources to nestmates (von Frisch 1967). Profitability of food sources is also communicated by the frequency of waggle runs during waggle dances (Seeley 1995). A colony will respond to waggle dance activity by allocating foragers to the advertised resource patch while decreasing foraging to others (Seeley 1995). Therefore, the waggle dance increases a colony’s foraging efficiency by directing recruits to rich floral patches when previously exploited resources become unprofitable (Gruter and Ratnieks 2011) and when resources are ephemeral in availability or patchily distributed over large areas (Seeley 1995; Sherman and Visscher 2002).

Since the decoding of the waggle dance language by von Frisch (1967), several studies have used the spatial information encoded in the waggle dance to study the foraging ecology of honey bees. Pioneering work by Visscher and Seeley (1982) used the waggle dance to map the spatial distribution of bee-visited resource patches in a temperate forest. They observed daily turnover in patch usage, indicating that colonies allocated foragers to few patches at any given time. Subsequent descriptive studies have reported similar findings but have also observed significant variation in patch distributions among the following: years (Beekman and Ratnieks 2000); colonies and habitats (Waddington et al. 1994); and, honey bee races (Schneider and Mcnally 1993).
Waggle dance activity is greater in complex landscapes (i.e. patchy forest and grassland habitats) than in simple landscapes (i.e. continuous agricultural habitats), suggesting that food collection may be enhanced through waggle dance communication in environments where floral resources are more patchily distributed (Steffan-Dewenter and Kuhn 2003).

The results of these studies illustrate the variable, dynamic nature of honey bee foraging ecology and laid the foundation for asking whether waggle dancing increases colony fitness. Waggle dancing most benefits colony food collection during periods of seasonal dearth when the abundance of food resources is low in the environment (Sherman and Visscher 2002; Okada et al. 2012). Dornhaus and Chittka (2004) expanded upon these findings by comparing food collection of colonies with and without waggle dance communication in tropical and temperate habitats. The elimination of waggle dance communication did not have a significant effect on food collection in temperate habitats. However, food collection was higher in colonies with waggle dance communication in tropical habitats, where floral resources can be patchily distributed through seasonal mass flowering of trees. As a whole, these findings show that the adaptive significance of the waggle dance is context dependent: that is, that the benefit of the waggle dance on food collection is a function of habitat specific characteristics such as the temporal availability and spatial distribution of resources in the environment.

The previously discussed studies have demonstrated significant variation in patch distributions, identified fitness benefits conferred by waggle dancing, and provided general insights into honey bee foraging ecology. However, our understanding of the diet and foraging ecology of honey bees in fragmented environments where native flowers and human subsidized, non-native flowers are available remains poor. Knowledge gained
from such a study could shed-light on the importance of non-native plants as a food source when native plants are seasonally unavailable, the role of introduced honey bees in pollinating native plants, and how honey bee foraging responds to anthropogenic change in general.

We therefore used the spatial information encoded in the waggle dance and DNA barcoding of bee-collected pollen to characterize the spatio-temporal foraging ecology of honey bees in La Jolla, California where native and non-native floral resources are available and are discretely partitioned into urban and natural habitat fragments. This environment is unique in that the Mediterranean climate is conducive to year-round foraging, but the relative abundances and spatial distributions of native and non-native flowers changes with season. We pose three questions. (1) Do foraging distances vary with season? (2) Do the spatial distributions of bee-visited floral patches vary with season? (3) What plants constitute the diet of honey bee colonies in our study area and are there seasonal patterns in host use?
Materials and Methods

Study site and colonies

We conducted our study at the University of California, San Diego Biological Field Station in La Jolla, California, from March 2010 to June 2010 and November 2010 to December 2011. The study site is situated in the California Floristic Province and possesses a true Mediterranean climate characterized by two seasons: warm, dry summers and cool, wet winters (Cowling et al. 1996). On average, the site receives ~ 300 mm of rainfall per year, typically from November to April. Wildflowers are most abundant in the environment during the short spring, growing season from late March to mid June. The study area is heavily developed, typical of urban Southern California. Native flora is often restricted to roadsides, small native habitat fragments, and natural preserves. The native flora of the study site is representative of a southern Californian coastal scrub community. Dominant native species include California sagebrush (*Artemisia californica*), coyote brush (*Baccharis pilularis*), lemonade berry (*Rhus integrifolia*), and Toyon (*Heteromeles arbutifolia*).

We used three colonies of the Italian honey bee (*Apis mellifera ligustica*), each housed in 3-frame observation hives whose internal entrance could be adjusted to direct foraging to only one side of the colony, thereby creating a preferred dance floor side. Bees were allowed to enter and exit the nest box through vinyl tubes leading to a nest entrance and could also exit the colony (when it opened) through a window in the temperature-controlled (30°C) observation room (32° 53’N, -117°13’W). Each colony contained approximately 1000-2000 workers.
Observing waggle dances and collecting pollen loads

To determine where pollen foragers were foraging and what plants they were visiting, we analyzed waggle dances for pollen and collected the pollen loads for DNA barcoding analysis. We observed each of the three different observation hives once per week for one hour between 0900 to 1500 hours, randomly beginning our observations when we observed any waggle dance activity. For each observation period, we opened the observation hive to allow filming and subsequent capture of waggle dancing pollen foragers. Bees were allowed to acclimate on the open comb for 5 minutes before data collection. Artificial lighting was directed away from the comb to prevent misdirection of dancers and recruits. A Panasonic PV-DV402D video camera was positioned approximately 1 m from the opened nest box to capture footage of waggle dancing pollen foragers. Bees recruiting for pollen sources typically dance before unloading their pollen loads (Visscher and Seeley 1982). Once we randomly selected a waggle dancing pollen forager, we noted the time, recorded 10-15 dance circuits (von Frisch 1967), and then captured the forager in a plastic vial, froze it, and subsequently removed the pollen loads with clean forceps into either 70 or 100% ethanol for subsequent identification.

Waggle dance footage was edited and analyzed on an Apple iMac computer. We define a waggle dance performance as the sequential production of multiple waggle dance circuits during a single recruitment bout. Only dance performances with at least four clearly viewable, consecutive waggle-circuits were decoded. Foraging distance to a bee-advertised floral patches was calculated according to the methods of Dornhaus and Chittka (2004). The compass direction to the floral patches were determined by calculating the average angle of the waggle phase relative to north and adding this value
to the angle of the sun’s azimuth at the time during which the dance was performed.

Floral patches locations decoded from waggle dances were plotted into a land-use map of our study area in ArcGIS v10 (ESRI, Redlands, California USA) by adding the northing and easting distance in meters of each of our points to the Universal Transverse Mercator (UTM) coordinate of the observation hives.

**Molecular Identification of pollen**

We treated 4 µl of pollen in alcohol solution with 10 µl of cyclohexane for 1 hour to digest the pollen exine coat (Doughty et al. 1993). After cyclohexane treatment, half of our samples were crushed under liquid nitrogen and DNA was extracted using the DNeasy Plant MiniKit (Qiagen 6910, Valencia, California USA). For the remaining samples, we used a modified direct pollen PCR (Polymerase Chain Reaction) protocol described by Petersen et al. (1996) for high-throughput recovery of PCR product. We switched our protocol halfway through our study to expedite the process of obtaining PCR products for sequencing. We added 14 µl of ddH2O and 5 µl of 10X PCR reaction buffer (Gennessee 42-400, San Diego, CA USA) to cyclohexane treated pollen and heated at 95°C for 30 minutes. The resulting solution was used as DNA template for PCR. DNA from multi-species pollen loads can yield PCR product, however, resultant sequences are often of short length and quality and are unusable for further analysis (Wilson et al. 2010). Wilson et al. (2010) found that even mixed pollen loads composed primarily of a single species (99 % w/v) were incapable of yielding sequence data. Because honey bees are floral constant and typically return to the hive with pure pollen
loads (Grant 1950), we believe that DNA barcoding is an appropriate method in identifying the important host plants honeybees use for pollen.

We performed PCR to amplify two plastid barcoding regions, *rbcLa* (~500 bp) and *matK* (~800 bp). The *matK* and *rbcLa* barcodes were amplified using the primers and protocols specified by the CBOL Plant Working Group (2009). The *matK* and *rbcLa* barcoding regions were amplified using primers 1R_KIM/3F_KIM (KJ Kim, unpublished) and rbcLa_F/rbcLa_R (Kress et al. 2009). The thermal cycling program for *matK* and *rbcLa* is as follows: initial denaturation at 95 °C for 4 min, 35 cycles at 94 °C for 30 seconds, annealing at gradient of 42–52°C for 45 seconds, 72 °C for 1 minute, and final extension at 72 °C for 10 minutes. For samples that failed to yield a *matK* amplicon, the *rbcLa* region was amplified. All PCR products were visualized on 0.8 % agarose gels. PCR products were purified with 0.08 µl Exonuclease I and 0.4 µl Shrimp Alkaline Phosphatase (USB Corporation 78201, Cleveland, OH USA) in 5 µl reaction volume. Samples were incubated at 37°C for 30 minutes then heated to 80°C for 15 minutes. Purified PCR products were sequenced using primers 3F_KIM and rbcLa_F for *matK* and *rbcLa* amplicons (Retrogen, San Diego, CA USA). Sequences were viewed and edited by eye in 4Peaks v 1.7.2 (Griekspoor 2006). Low quality bases (PHRED score < 15) and ambiguous peaks were scored as “N”.

We performed BLASTn searches in Genbank to identify pollen sequences. BLAST scores $\geq$1000 and $\geq$ 97% identity for *matK* sequences and $\geq$900 and $\geq$ 99% for *rbcLa* sequences were considered putative matches to genus and in some cases, species. When pollen could not be identified to species, we used a comprehensive list of species found in the study area to determine the most probable species identity. The data used to
generate this list was obtained from herbaria records from the San Diego County Plant
Atlas (http://www.sdplantatlas.org) and the field guide San Diego County Native Plants
(Lightner 2011). For pollen samples for which there were several species within a genus
that were possible matches, we obtained museum curated voucher specimens of several
species within that genus from the herbarium collection at the San Diego Natural History
Museum and generated sequence data for these vouchers using the methods described
above for pollen. However, for voucher specimens, we obtained reverse and forward
sequences and generated contiguous sequences for them in CodonCode Aligner v4.0
(Codon Code Corporation, Dedham, MA, USA) We used MEGA v5.05 (Tamura et al.
2011) to align sequences from pollen, voucher, and Genbank accessions for all species
found in San Diego in a given family. Neighbor-Joining trees were generated using the
Kimura 2 Parameter model and 1000 bootstrap replicates (de Vere et al. 2012). The
formation of monophyletic clades containing pollen and known voucher or Genbank
accession sequences with ≥ 50 % bootstrap support were considered putative matches to
species for pollen.

Statistical Analyses

Analyses were performed using JMP v10 statistical software. We used 30 years
(1981 – 2010) verified climate records collected at Lindbergh Field (sensor CA-SD-37)
from the NOAA National Climate Center to determine seasonality in the study area. We
delineated seasons based on the natural patterns in rainfall, cooling, and warming in the
study area (Fig. 1). Using this method, we coded each month as belonging to “dormant,
growth, and dry” seasons. The wet, cool months from November through March were
considered to be the “dormant” season. The months that experienced simultaneous warming and drying from April through June were considered to be the “growth” season. The dry, warm months from July through October were considered to be the “dry” season (Fig. 1). A Kruskal-Wallis test revealed mean daily high temperatures were significantly different across seasons (Kruskal-Wallis test: $H = 288.52$, $p < 0.0001$).

We pooled data across colonies to detect general patterns in honey bee foraging ecology. Foraging distance data were log$_{10}$-transformed to achieve normality. To determine whether honey bee foraging distances vary with season, we used a one-way ANOVA to compare foraging distance across seasons. We used a post-hoc Tukey Honest Significant Difference (HSD) tests to determine if mean foraging distances varied significantly by season. We used logistic regression to determine whether foraging distance was correlated with monthly mean daily high temperature.

To investigate how the spatial distribution of bee-visited floral patches varied with season, we used the R package SPATSTAT v1.25-4 (Baddley 2005a) to calculate Clark and Evans (1954) index of dispersion, $R_D$, for all bee-visited floral patches decoded from waggle dances for each season. An $R_D$-value of 1 indicates a uniform distribution of patches. Values of $R < 1$ indicate a clumped distribution; $R_D > 1$ indicates hyperdispersion of patches (Dornhaus and Chittka 2004). We calculated $R_D$ using a 1.0 x $10^8$ m$^2$-window size based on the minimum and maximum northing and easting distances decoded from waggle dances. We applied Donnelly’s (1978) correction to correct for edge effects to account for our use of a rectangular window. We also calculated $R_D$ for each month of floral-patch data. When we did not have enough data in a month (due to low foraging in winter months) to calculate a robust value of $R_D$, we calculated a single
value of $R_D$ for a given set of months. We did this for October through December 2010; January through March 2011; September and October 2011. We removed two points in our analysis that were found to be in the Pacific Ocean. We used logistic Regression to determine whether spatial distribution of bee-visited floral patches was correlated with monthly mean daily high temperature.

To detect seasonal variation in forage plant usage, we used a $\chi^2$-test of association to determine if the proportion of native to non-native pollen found in corbicula pollen loads varied with season.
Results

In total, we decoded 235 waggle dance performances from three observation colonies over 17 months of data collection and 4 seasonal replicates (Fig. 2; Fig. 5). On average we observed 11.88 ± 7.81 dance circuits per waggle dance performance. The mean foraging distance for the entire dataset was 1217.5 ± 1138.0 m (median = 928.7 m).

Effect of season on foraging distances

Mean foraging distances were 640.0 ± 618.5 m in the dormant season (n = 28), 1044.3 ± 1011.3 m in the growth season (n = 133), and 1747.4 ± 1301.3 m in the dry season (n = 74). There was a significant effect of season (Fig. 3A; ANOVA, $F_{2,232} = 19.09$, $p < 0.0001$). Foraging distance varied significantly between each season (Tukey HSD, $\alpha = 0.05$, $Q = 2.36$). There was a significant positive relationship between monthly mean daily high temperature and monthly logged mean foraging distance (Fig. 3B; ANOVA, $F_{1,14} = 9.51$, $p = 0.008$, $R^2 = 0.51$). On average, foragers flew the shortest distances during the dormant season and increased flight distances as ambient temperatures rose with season.

Effect of season on foraging spatial distribution

The mean value of $R_D$ across our study was 0.38 ± 0.18 (n = 10). This value of $R_D$ is lower than 1, suggesting that rich, bee-visited floral resources are clumped and not uniformly distributed in our study site. Seasonal measurements of $R_D$ are as follows (Fig. 4): dormant season ($R_D = 0.24$); growth season ($R_D = 0.41$); dry season ($R_D = 0.60$). The highest monthly value of $R_D$ was noted in June 2010 (Fig. 5; $R_D = 0.58$). The lowest
monthly value of $R_D$ was noted in May 2010 (Fig. 5; $R_D = 0.18$). We found no significant correlation between $R_D$ and monthly mean daily high temperature ($F_{1,8} = 1.76$, $p = 0.22$). The spatial distribution of bee-visited floral patches varied with season. Bees focused their foraging efforts for pollen on few, clumped patches during the dormant season performed increasingly wider searches for pollen with changes in season.

**Effect of season on the use of native and non-native plants**

We successfully extracted, amplified, and sequenced DNA barcodes from 82% (217 out of 263) of corbicular pollen samples. Mean length of sequences was $715 \pm 5.7$ b.p. for *matK* ($n = 187$) and $526 \pm 14.1$ b.p. for *rbcLa* ($n = 30$). In total, 32 families, 55 genera, and ca. 100 species were represented in our dataset. We detected a significant difference in the proportion of pollen loads with native or non-native pollen with season (Fig. 6, LRT $\chi^2 = 19.09$, $p < 0.001$). Bees primarily collected pollen from native shrubs during the dormant season; equal proportions of native and non-native herbs and shrubs during the growing season; and mainly non-native trees during the dry season (Table 1).
Discussion

We used the waggle dance and DNA barcoding to characterize the spatio-temporal foraging ecology of honey bees in a fragmented environment. We found that honey bees altered their foraging behavior over the course of several seasons to exploit common native and non-native plant species for pollen. Foragers, on average, flew 1.5 and 2.8 times further during the growing and dry seasons than in the dormant season (Fig. 3A). Moreover, there was a strong, positive correlation between monthly mean daily high temperature and foraging distance (Fig. 3B). Bee-visited floral patches were most clumped in the environment during the dormant season and became more dispersed with seasonal progression (Fig. 4). Using DNA barcoding, we found that bees collected pollen from a wide variety of plant species, but repeatedly foraged on a subset of common species (Table 1). Furthermore, we detected significant turnover in the proportion of native to non-native pollen collected by foragers with season indicating that bees tune their foraging efforts to the flowering phenology of common native and non-native plants (Fig. 6). Foragers mainly collected native pollen during the dormant season, equal proportions of native and non-native pollen during the growing season, and mostly non-native pollen during the dry season.

Taken together, our results show that honey bees repeatedly recruit to and collect native pollen from scrub fragments during the cooler seasons and collect pollen from more widely dispersed, non-native species during warmer seasons of the year, thus indicating that honey bees can change their foraging behavior with season to focus their foraging efforts on common native and non-native plants. Our results also have
interesting implications regarding the role of introduced honey bees in pollinating native plants in increasingly fragmented landscapes.

Our study is, to our best knowledge, the first to use DNA barcoding to study the pollen foraging ecology of a highly social, generalist pollinator. Wilson et al. (2010) were the first to apply molecular methods to characterize the pollen foraging ecology of a solitary, native, specialist pollinator in a depauperate habitat in Hawaii. Through genetic analysis, they were able to show that Hawaiian *Hyleus* bees collected pollen from a surprisingly small number of plants. Their findings were in contrast to published reports that observed *Hyleus* interacting with more species, indicating that molecular palynology may not capture the true breadth of pollinator interactions. However, the use of molecular methods in identifying bee-collected pollen can supplement floral visitation records to identify the important plant species bees strongly interact with, because bees must visit flowers of a given species numerous times to collect sufficient pollen to provision larvae. The strength of a given interaction can be quantified by counting the number of specimens carrying pollen of a given species in pollen-carrying structures (e.g. corbiculae, scopae, crops) or determining the volume of pollen in nests that are of a given species. This study shows the utility of DNA barcoding methods in studying plant-pollinator interactions in that it obviates the need rely upon traditional light microscopy methods that are time consuming and require the amassing of a large library of reference slides, a process that may not be tractable to the study native pollinators in especially biodiverse habitats or the study of highly social, super-generalist pollinators such as honey bees.
Effect of season on foraging distances

Average foraging distance across seasons for our study was 1128 m. Our measurements are similar than those reported by Waddington et al. (1994) who reported mean foraging distances of 534 – 1138 m in a similar southern California suburban habitat. In contrast, studies performed in other habitats have noted far greater mean foraging distances than those reported in our study (Visscher and Seeley 1982; Beekman and Ratnieks 2000; Steffan-Dewenter and Kuhn 2003). The high degree of variance in foraging distances across these studies further supports the argument that how honey bees forage is dependent on ecological and environmental conditions specific to a given habitat.

We detected significant differences in foraging distance with season. Moreover, we found a significant positive relationship between monthly mean daily high temperature and foraging distance. One possible explanation for this may be that flight distance is a function of ambient temperature and physiological constraints on flight. Honey bees are endothermic poikilotherms and have evolved a variety of physiological and behavioral contrivances by which they actively maintain body temperature during flight (Heinrich and Esch 1994). Prolonged flight may be metabolically costly at lower (< 19°C) and higher (> 40°C) ambient temperatures because more energy must be expended to maintain constant temperature in flight muscles (Cooper et al. 1985; Woods et al. 2005). In our study area, daytime temperatures were rarely above or below ambient air temperatures that limit flight in honey bees, suggesting that our observed seasonal variation in foraging distances is not strongly influenced by thermal constraints on flight.
Effect of season on foraging spatial distribution

We believe that seasonal variation in foraging distances may be attributed to differences in the temporal and spatial distribution of profitable, bee-visited floral resources across seasons. Other studies show that foraging distances increase during periods of dearth or in response to mass flowering of distant, patchy resources (Schneider and McNally 1993; Beekman and Ratnieks 2000). We observed an increase in mean foraging distances with season and saw that foragers flew furthest during the dry season (Fig. 3A), which is a period of seasonal low rainfall in southern California (Miller et al. 1983). The spatial distribution of exploited floral patches (Fig. 4) is most clustered during the dormant season (Fig. 4; $R_D = 0.24$), becoming more uniformly distributed in the study area during the growing season (Fig. 4; $R_D = 0.41$) and most dispersed during the dry season (Fig. 4; $R_D = 0.60$). We were unable to formally test whether patchiness of resources varied with season due to a lack of data available for the dormant season which we attribute to normal, seasonal fluctuations in worker numbers (Seeley 1995). However, our seasonal measurements of $R$ indicate that there are quantifiable, seasonal differences in how honey bees interact with the landscape to forage for pollen.

Effect of season on the use of native and non-native plants

The identification of bee-collected pollen through DNA barcoding showed that though foragers collected pollen from a wide variety of species, foraging was focused on relatively few species during any given season (Table 1). During the dormant season months, mean foraging distances were relatively short, and the majority of pollen was collected from *Rhus integrifolia*, a native, winter-flowering shrub. During the growth
season months, foraging distances increased and foragers repeatedly collected pollen from seven abundant, widely distributed, spring-flowering native shrubs and invasive herbs (Table 1). During the dry season, we observed the greatest mean foraging distances and found that the majority of pollen was collected from *Baccharis pilularis*, a native shrub, and several non-native Eucalypt species. Thus, introduced plants provided an important protein subsidy to honey bee colonies during the dry season.

During the dormant and dry seasons, the abundance of flowers in the environment is markedly lower than during the growing season when native and non-native annuals are in bloom; therefore bees will opportunistically recruit to whatever species are flowering during that time. In the case of the dormant season, it is not surprising to see that colonies allocated foragers to *R. integrifolia*, which forms dense stands in the scrub canyons flanking the area where our colonies were housed. Furthermore, it is not surprising to see that colonies primarily collected from *B. pilularis* and several eucalyptus species during the dry season. *Baccharis pilularis* is a fall-flowering shrub that is common in coastal scrub habitats throughout southern California. Eucalypts are widely distributed all over San Diego County and can be found in dense stands throughout the study area. Both *B. pilularis* and eucalypt species are often the only flowering plants available to bees during the drier, warmer parts of the year.

During the growing season, when native and non-native annual flowers were most abundant at our site, bees predominantly foraged on a small subset of flowering species (Table 1). It has been suggested that waggle dancing benefits colonies in habitats with many flowering species by directing foraging efforts to only the most profitable resources (Donaldson-Matasci and Dornhaus 2012). Identification of pollen collected during the
Growing season provides direct empirical support for this hypothesis in that the preferred plant species are abundant in the environment and flower en masse during this time. Considering the “super-generalist” habit of honey bees, we were surprised to see how few species our colonies repeatedly exploited for pollen despite our study area being situated in a national and global hotspot of biodiversity (Dobson et al. 1997; Myers et al. 2000). Our study largely focused on pollen sources that foragers recruited for and were therefore deemed valuable for the colony. It is possible that bees collected pollen from a broader range of species, but it is nonetheless interesting that so few species, in seasons of dearth and plenty, were the focus of colony foraging efforts and were therefore, for bee-pollinated species, the beneficiaries of pollination.

The fact that our colonies repeatedly foraged for pollen from a small subset of available flowering species at any given time indicate that though honey bees may not be acceptable substitutes for pollinating highly specialized, rare native plants in our study area, they may serve as reliable pollinators for common native species. The importance of honey bees as pollinators of native plants is disputed (Potts et al. 2006; Aizen et al. 2008; Kaiser-Bunbury et al. 2009). A recent meta-analysis has shown that pollinator densities and diversities are significantly adversely affected in heavily fragmented habitats (Winfree et al. 2009). Therefore in heavily disturbed habitats such as ours, honey bees may play an important role in pollinating common native plants when native pollinators become extirpated. The molecular methods employed in our study could be used in future research to characterize the diet and ecology of understudied native pollinators. Such knowledge could be used to better understand the consequences of native pollinator extinctions on pollinator networks in an increasingly fragmented, urbanized world.
**Figures and Tables**

**Figure 1:** Monthly change in mean daily high temperature (°C) and rainfall. Long-term daily normal data was collected by NOAA from Lindbergh Field (Station ID: GHCND: USW00023188) San Diego, California. Data was collected from 1981 to 2010.
Figure 2: The location of all bee-visited forage patches in the study area inferred from the waggle dance. The location of bee-visited forage patches was plotted onto a land-use map of the study area in La Jolla, California (LANDUSE-CURRENT, 2009; SANDAG, San Diego, California, USA). White areas indicate urban, developed areas. Green areas denote open habitat fragments. Locations of bee foraging patches decoded from waggle dances are denoted by red dots. Observation hives were housed at the location denoted by the orange star.
Figure 3: Effects of season and ambient temperature on foraging distances. (A) Effects of season on foraging distances obtained from decoding waggle dances. Mean (± SE) bee foraging distances (m) for each season. (B) Scatter plot and regression of monthly mean bee foraging distances (m) by monthly mean daily high temperature (°C). Each shape denotes the season from which the monthly data was calculated: Diamond (Dormant); Circle (Growth); Square (Dry). Data was pooled across all observation colonies.
Fig. 4: Density maps of bee-visited forage patches decoded from waggle dances for each season. Window size was set to $1.0 \times 10^8 \text{ m}^2$. Warmer colors denote a higher density of points at a $100 \text{ m}^2$ spatial resolution. Data was polled across all observation colonies.
Figure 5: Monthly change in $R_D$ (Index of Dispersion) from May 2010 to October 2011. Data was pooled across all observation colonies.
Figure 6: Proportion of pollen loads identified collected from native or non-native sources for each season. Open bars represent the proportions of pollen loads in any given season identified containing native flower species. Black bars represent the proportions of pollen loads in any given season identified containing native flower species. Data was pooled across all observation colonies.
Table 1: Common species identified through DNA barcoding in bee-collected pollen loads. Species represented on this table were identified in $\geq 5\%$ of all pollen loads collected within any given season. Data was pooled across all observation colonies.
<table>
<thead>
<tr>
<th>Season</th>
<th>Family</th>
<th>Identity</th>
<th>Habit</th>
<th>Status</th>
<th>Proportion of Loads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dormant</td>
<td>Anacardiaceae</td>
<td><em>Rhus integrifolia</em></td>
<td>Perennial shrub</td>
<td>Native</td>
<td>0.71</td>
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<tr>
<td>Growth</td>
<td>Anacardiaceae</td>
<td><em>Toxicodendron diversilobum</em></td>
<td>Perennial vine</td>
<td>Native</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Asteraceae</td>
<td><em>Centaurea melitensis</em></td>
<td>Annual herb</td>
<td>Non-Native</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Brassicaceae</td>
<td><em>Brassica nigra</em></td>
<td>Annual herb</td>
<td>Non-Native</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Myrtaceae</td>
<td><em>Eucalypts</em> (multiple)</td>
<td>Tree</td>
<td>Non-Native</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Polygonaceae</td>
<td><em>Eriogonum fasciculatum</em></td>
<td>Perennial herb</td>
<td>Native</td>
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<tr>
<td></td>
<td>Rosaceae</td>
<td><em>Adenostoma fasciculatum</em></td>
<td>Perennial shrub</td>
<td>Native</td>
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</tr>
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<td></td>
<td><em>Heteromeles arbutifolia</em></td>
<td>Perennial shrub</td>
<td>Native</td>
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<tr>
<td>Dry</td>
<td>Asteraceae</td>
<td><em>Baccharis spp.</em></td>
<td>Perennial shrub</td>
<td>Native</td>
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<tr>
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<td>Myrtaceae</td>
<td><em>Eucalypts</em> (multiple)</td>
<td>Tree</td>
<td>Non-Native</td>
<td>0.27</td>
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<tr>
<td></td>
<td></td>
<td><em>Meleleuca viridiflora</em></td>
<td>Tree</td>
<td>Non-Native</td>
<td>0.11</td>
</tr>
</tbody>
</table>
References


Griekspoor A, Groothuis, Tom (2006) 4Peaks. In, 1.7.2 edn. mekentosj.com


