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RESEARCH ARTICLE

Seasonal trends in honey bee pollen foraging revealed through DNA barcoding of bee-collected pollen

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Abstract Recent declines in the abundance and diversity of wild bee species have made it important to understand how introduced species such as honey bees contribute to pollinating native plants. In this study, we decoded the waggle dances of *Apis mellifera* pollen foragers and used DNA barcoding of bee-collected pollen over 17 months to characterize spatio-temporal patterns in recruitment behavior and dietary breadth in a diverse yet fragmented Mediterranean scrub environment. Foragers danced for small, nearby patches of pollen resources during the cool *dormant* season but danced for 1.6–2.7-fold more distant and more widely dispersed resources during the hot *dry* season, a time of seasonal drought and floral dearth. Individual foragers collected pollen from a wide variety of species, but colonies focused recruitment on a small subset of common species in flower during each season: 2.7, 6.3, and 10% of all species in bloom during the *dormant*, *growth*, and *dry* seasons, respectively. Pollen foragers danced almost exclusively for cold-adapted native species during the *dormant* season, equally for native and non-native plants during the short *growth* season, and primarily for non-native ornamental species during the *dry* season. Our results suggest that honey bee recruitment behavior and dietary breadth may be influenced by seasonal changes in pollen resources. Furthermore, they indicate that honey bees may be useful in pollinating common native plants, and that non-native ornamental species can provide a major source of colony protein during a seasonal dearth of flowering native species.

Keywords Pollen · Foraging · Seasonal · DNA barcoding

Introduction

The abundance and diversity of native pollinators has globally declined in multiple ecosystems (Biesmeijer et al. 2006; Memmott et al. 2007; Winfree et al. 2009; Potts et al. 2010a; Burkle et al. 2013). Although honey bee populations have experienced some declines (e.g., Oldroyd 2007; Potts et al. 2010b; Breeze et al. 2011), they are present in disturbed and natural habitats throughout the world and can provide significant pollination services (Watanabe 1994; Aizen and Harder 2009) even where they are not native (Goulson 2003). Although honey bees can compete with native bees for floral resources (reviewed in Paini 2004), they can also effectively pollinate native plant species where they have been introduced (reviewed in Goulson 2003). Honey bees may therefore bolster community-level pollination services in areas where native pollinators have declined. Understanding honey bee foraging ecology, particularly how this changes seasonally among native and non-native plants, is therefore important.

The adaptability of honey bee colonies to a wide variety of environmental conditions has contributed to their occurrence in temperate and sub-tropical habitats throughout the world (Michener 2000). Two traits likely contribute

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to this success: their super-generalist ability to forage on a broad range of floral species (Richardson et al. 2000) and the waggle dance, a complex behavior in which nestmates direct each other to profitable food resources by communicating its direction and distance from the nest (von Frisch 1967). Although food is seasonally variable and often patchy, the waggle dance helps mitigate this spatial and temporal heterogeneity because it allows the colony to efficiently adapt and reallocate its foraging (Beekman and Bin Lew 2008). Experimental inhibition of waggle dancing or reducing waggle dance information has been found to reduce food collection (a proxy for colony fitness) when floral resource availability varies in time (Sherman and Visscher 2002; Dornhaus and Chittka 2004; Okada et al. 2012), space (Dornhaus and Chittka 2004), or quality (Donaldson-Matasci and Dornhaus 2012).

Decoding waggle dances has enabled researchers to understand how colonies allocate foragers over the landscape (Balfour et al. 2015; Garbuzov et al. 2015a, b). Visscher and Seeley (1982) showed that colonies in a temperate forest had a wide foraging range (up to 6000 m) and could rapidly adjust their foraging in response to short-term, daily changes in resource profitability. Other studies have extended this approach to different habitats (Dornhaus and Chittka 2004; Donaldson-Matasci and Dornhaus 2012) and seasons, such as summer in a temperate New York forest (Visscher and Seeley 1982), spring in Florida and winter in California (Waddington et al. 1994), autumn in Japan (Okada et al. 2012), the *dry* season in Costa Rica (Schneider and Hall 1997), spring and summer in Great Britain (Beekman and Ratnieks 2000), late spring to early summer in Germany (Steffan-Dewenter and Kuhn 2003), and spring in Botswana (Schneider and McNally 1993).

In general, colonies increase their foraging distance when food becomes scarcer (Schneider and McNally 1993). Couvillon et al. (2014) measured foraging distances for nectar sources over 2 years in a temperate habitat, the British countryside, and found that mean foraging distances were shortest during the spring and greatest during the summer. Danner et al. (2016) similarly showed that honey bee foraging distances decreased during a seasonal period of food abundance. These studies confirmed that colonies search and forage over wide areas and adjust this foraging in response to short (daily and weekly)- and long (monthly)-term fluctuations in resource availability. However, additional long-term data from other habitats will help confirm these general patterns and increase our overall understanding of how honey bee recruitment responds to seasonal food dearth.

Because of the pollination service that honey bees provide (Watanabe 1994; Aizen and Harder 2009), the proportion of species that they visit out of available species, diet breadth, is important. Analysis of pan-trap-collected pollen, which samples all pollen entering the nest, demonstrates that colonies collect pollen from many species (Baum et al. 2004; Dimou and Thrasyvoulou 2007) but focus on a subset of available plants at any given time (Donaldson-Matasci and Dornhaus 2014). Similarly, although workers collect pollen from a wide variety of plant species, the colony may focus recruitment for a more limited subset of plant species during different seasons. To date, this "recruitment" diet breadth has not been examined over multiple seasons. However, this information is of interest because recruitment dancing plays a key role in colony decision-making about what resources are important to exploit (Dyer 2002).

Finally, there is debate about the importance of honey bees as pollinators of native and non-native plant species, and what role they may play in the maintenance of biodiversity in light of global declines in honey bee populations and native ecosystems (Aebil et al. 2012; Ollerton et al. 2012). Understanding seasonal patterns in recruitment behavior and dietary breadth can shed light on how honey bees interact with both native and non-native plants (Schweiger et al. 2010) and illuminate their contribution to pollinating native plants.

Our main goal was therefore to determine the seasonal patterns in colony recruitment dancing for pollen from native and non-native plants. We focused on pollen because it is the primary source of colony protein (Seeley 1995). Each week over 17 months, we monitored three colonies, mapped the locations of pollen resources inferred from waggle dancing, and used DNA barcoding (Galimberti et al. 2014; Bell et al. 2016) of dancer pollen loads to determine the plant species that they recruited for. We chose a study site in southern California that has a diverse native plant community and a mild Mediterranean-type climate that allows year-round bee foraging but has discrete seasons during which the spatial distribution, abundance, and species identity of flowering native and non-native plants markedly change. To estimate available floral species, we analyzed records from the San Diego County Plant Atlas and the CalFlora database. Based upon these data, we expected that important pollen sources would be more abundant, diverse, and uniformly distributed during the growing season and become patchier and scarcer during the *dry* season. In addition, these records suggested seasonal differences in the abundance of flowering native and non-native plants. We therefore predicted seasonal variation in the following: (1) foraging distances to bee-advertised food resources, (2) the spatial distribution of bee-advertised food resources, and (3) recruitment for pollen from native and non-native plants.

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 (32°53′N, −117°13′W), from March 2010 to June 2010 and November 2010 to December 2011. Honey bees are not native to this region, but are commonly found (Kono and Kohn 2015). Ecologically, our study site is in the California Floristic Province and possesses a true Mediterranean climate characterized by warm, dry summers and cool, wet winters (Cowling et al. 1996; Ackerly 2009). On average, the area receives 300 mm of rainfall per year, mostly during November to April. In southern California, many native species flower in the spring growing season from late March to mid-June, while many non-native species (invasive and ornamental) flower outside of this time due to water subsidization of garden plants or phenological differences (Godoy et al. 2009). The native flora of our study site is representative of a southern Californian coastal scrub community and is often restricted to roadsides, small native habitat fragments, and natural preserves. Common non-native species include various species of *Eucalyptus* and invasive herbs such as *Brassica nigra* (Bell and Muller 1973). We conducted our study at a single site because we wished to collect detailed data on pollen recruitment and monitor multiple colonies weekly over a year. At our field site, colonies do not exhibit strong overwintering and can produce some brood even in the winter months.

We simultaneously monitored three colonies of the Italian honey bee (*Apis mellifera ligustica*). Colonies originated as package bees (CF Koehnen & Sons, Glenn, CA, 95943 USA) with Koehnen queens. Each package was placed in a standard Langstroth ten-frame hive and allowed to gain strength for 10 months before three combs were selected (two combs with good brood density and pollen provisions and one honey comb) per colony for placement into three-frame observation hives. All colonies were in good health. The original queens were also transferred into the observation hives. These observation colonies were not re-queened for the duration of the experiment. Each observation hive had an internal entrance that was adjusted to direct foraging to only one side of the colony, thereby creating a preferred dance floor side and allowing us to monitor all recruitment dances (Nieh 2010). Bees were allowed to enter and exit each observation hive through a 4-cm-diameter and 60-cm-long vinyl tube connected to the nest entrance and could also exit the colony through a window (when it was opened) in the temperature-controlled (30 °C) observation room. We observed no robbing of our observation colonies during this experiment and found that even wasp predators (*Vespula pensylvanica*) did not enter the observation colonies. It is possible that the extended tube entrance facilitated colony defenses.

To determine colony size, we took digital images of an approximately 66 cm^2 area of comb where the distribution of bees corresponded to the overall distribution of bees on both sides of all three combs on days when we collected data (approximately once per week per colony). We counted the number of bees in this comb area, defining a single bee as an individual with >50% of its body within the visible frame. We then calculated the precise area covered by our frame using the width of multiple worker cells (5.2 mm per cell, Spivak and Erickson 1992) to calibrate image size. We then scaled our estimate to the total comb area of the colony (5760 cm^2 for both sides of three combs) to obtain the total number of bees.

Observing waggle dances and collecting pollen loads

To determine the location and identity of pollen resources that the colony recruited for, we video recorded randomly selected waggle dancing bees with pollen loads in their corbiculae and then collected the pollen loads for DNA barcoding. We observed each of the three hives approximately once per week for 1 h between 0900 and 1500 h, randomly choosing the hour for a given hive and beginning our 1-h observation period when we observed waggle dance activity. This sampling method focused only on pollen sources that honey bees would recruit for between 0900 and 1500 h. It did not capture pollen sources that non-*Apis* bees foraged for, that were only available at different times, or that were not considered sufficiently profitable to merit recruitment by the bees.

For each observation period, we removed the clear cover over the combs to permit filming and subsequent capture of waggle dancing pollen foragers. Bees were allowed to acclimate on the open comb for 5 min before data collection. To avoid altering dancer orientation, we used relatively dim light directed away from the combs. This light did not alter dancer behavior, as determined by examining the orientation of dancers in the near dark and under lighting. A Panasonic PV-DV402D video camera was positioned approximately 1 m from the combs to videotape waggle dancing. Bees recruiting for pollen sources typically dance before unloading their pollen loads (Visscher and Seeley 1982). We randomly selected a waggle dancing pollen forager, focused in on this dancer, noted the time of day, recorded 10–15 waggle runs (von Frisch 1967), captured the forager in a plastic vial, froze it, and removed the pollen loads with clean forceps into ethanol for subsequent identification. We therefore observed each dancing forager only once.

We imported, edited, and analyzed waggle dance footage in iMovie v. 9.0.2 (Apple Inc. 2010). We define a waggle dance performance as the sequential production of multiple waggle dance circuits (von Frisch 1967) during a single recruitment bout. There is some error in the spatial coordinates specified by honey bee waggle dances (Towne and Gould 1988; Okada et al. 2014). However, averaging multiple waggle phase durations and angles reduces this error (von Frisch 1967). Only dance performances with at least four clearly viewable, consecutive waggle runs were decoded and included in our analyses. Fortunately, spatial encoding errors in the waggle dance decrease with increasing distance to the advertised location (Weidenmuller and Seeley 1999; Towne and Gould 1988). This phenomenon decreases the accuracy problem. In addition, we used commonly employed methods to calculate distance (method of Dornhaus and Chittka 2004) to ensure that our results can be directly compared with the results of other published papers.

We calculated the compass direction to floral patches by measuring the average waggle phase angle and determining the sun's azimuth (NOAA Solar Calculator, http://www.esrl.noaa.gov/gmd/grad/solcalc/) when the dance was performed (von Frisch 1967). Floral patch locations were then plotted onto a land-use map with ArcGIS v10 (ESRI, Redlands, California USA) by adding the north–south and east–west distance in meters of each of our points to the Universal Transverse Mercator (UTM) coordinate of the observation hives. Throughout this paper, we use the terms "foraging distance" and "foraging spatial distribution" to refer to the spatial location of natural pollen sources that colonies recruited for.

Molecular pollen identification

We placed 4 µl of pollen in alcohol solution with 10 µl of cyclohexane for 1 h to digest the pollen coat (Doughty et al. 1993). After cyclohexane treatment, half of our samples were frozen with liquid nitrogen and crushed. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen 6910, Valencia, California USA). We switched our protocol halfway through our study to expedite the process of obtaining PCR products for sequencing. 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. To all samples, we added 14 µl of ddH_2O and 5 µl of 10× PCR reaction buffer (Genessee Scientific, 42-400, San Diego, CA USA) to thoroughly mixed cyclohexane-treated pollen loads, heating at 95 °C for 30 min. The resulting solution was used as a DNA template for PCR. Wilson et al. (2010) found that mixed pollen loads composed of multiple species (as low as 1% w/v) did not yield usable sequence data because the resulting sequences were very short and of low quality. Moreover, honey bees are floral constant and typically return to the hive with pure pollen loads (Grant 1950). We therefore believe that DNA barcoding is an appropriate method to identify the important host plants that honey bees 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* consisted of initial denaturation at 95 °C for 4 min, 35 cycles at 94 °C for 30 s, annealing at a gradient of 42–52 °C for 45 s, 72 °C for 1 min, and final extension at 72 °C for 10 min. For samples that failed to yield a *matK* amplicon, we amplified the *rbcLa* region. 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 min and then heated to 80 °C for 15 min. 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 (http://mekentosj.com/4peaks/). 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 ≥1000 and ≥97% identity for *matK* sequences and ≥900 and ≥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 native and non-native species found in the study area to determine the most probable species identity. The data used to generate this list were obtained from herbaria records from the San Diego County Plant Atlas database (http://www.sdplantatlas.org) and the field guide San Diego County Native Plants (Lightner 2011).

For pollen samples identified to genus but with multiple species matches, we obtained museum-curated voucher specimens of several species within that genus from the herbarium collection at the San Diego Natural History Museum (2008) and sequenced the vouchers as described above. However, for voucher specimens, we obtained reverse and forward sequences and generated contiguous sequences using 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 available 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 was considered a putative match to determine pollen species.

Seasonal variation in flowering plant species

To determine if the flowering of native and non-native plants varied with season, we generated a species list of all plants in our study system using herbarium records from the San Diego County Plant Atlas database (http://www.sdplantatlas.org). We determined the months in which these species flowered using flowering phenology data from the CalFlora database (http://www.calflora.org/). These phenology data are coded in months, not seasons, and we therefore coded species that flowered >50% of the time during the months comprising a particular season. In total, we used 4048 collection records on 540 native and 275 non-native flowering species from 410 different genera and 94 families.

Statistical analyses

Analyses were performed using JMP v10 statistical software. We used 30 years (1981–2010) of verified climate records collected at Lindbergh Field (sensor CA-SD-37, Station ID: GHCND: USW00023188) 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. 1a). Using this method, we coded each month as belonging to *dormant*, *growth*, and *dry* seasons. We defined the *dormant* season as the wet and cool months from November through March when many numerically dominant native plant species have not yet begun flowering. The months that experienced simultaneous warming and drying from April through June were considered the *growth* season. We define the *dry* season as the dry and warm months from July through October when drought-adapted native species and many human-subsidized non-natives flower (Fig. 1a). A Kruskal–Wallis test revealed that mean daily high temperatures were significantly different across seasons (Kruskal–Wallis test: $H_2 = 288.52$, $p < 0.0001$).

We analyzed the effect of season on waggle dancing with a Kruskal–Wallis test and post hoc Steel–Dwass tests. To determine if honey bee foraging distances vary with season, we used ANOVA to test the effects of colony (random effect, REML algorithm) and season (fixed effect) on foraging distances. Foraging distance data were $\log_{10}$ transformed to achieve normality. We used a post hoc Tukey Honest Significant Difference (HSD) test to determine if mean foraging distances varied significantly by season. Both temperature and precipitation are important climate variables in Mediterranean-type plant communities (Ackerly 2009). Seasonal patterns of precipitation in Mediterranean-type habitats are generally predictable, with rainfall events typically occurring in the cooler parts of the year. However, the amount and precise timing of rainfall can vary markedly from year to year (Gasith and Resh 1999). As such, we found rainfall data to be much more variable and statistically noisy at both short and long temporal scales in our study site. We therefore used logistic regression to determine whether foraging distance was correlated with monthly mean daily high temperature.

To determine how the spatial distribution of bee-visited floral patches varied with season, we used the command clarkevans.test in the package SPATSTAT v1.25-4 (Baddley and Turner 2005) in R v2.15.1 to calculate Clark and Evans (1954) index of dispersion, R_D, and to test for complete spatial randomness (CSR) 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_D <1 indicate clumping and R_D >1 indicates hyperdispersion (Dornhaus and Chittka 2004). We calculated R_D using a 1.0×10^8 m^2 window size based on the minimum and maximum northing (north–south) and easting (east–west) distances decoded from waggle dances relative to the location of the observation hives. We applied Donnelly (1978) correction to correct for edge effects to account for our use of a rectangular window. Furthermore, we generated a null distribution by conducting 1000 Monte-Carlo replicates for each seasonal spatial point pattern to test whether they deviated significantly from complete spatial randomness. We also calculated R_D for each month of floral patch data. When we did not have enough data (averaging ≤2 patch locations per week in a month, due to low foraging activity in winter months or extended inclement weather), we calculated a single value of R_D over a set of months to obtain a robust R_D value. We did this for October through December 2010, January through March 2011, and September and October 2011.

We used logistic regression to determine if the spatial distribution of bee-visited floral patches correlated with monthly mean daily high temperature. To determine if the proportion of native to non-native pollen collected by dancers varied with season, we used Likelihood Ratio (LR) contingency analysis. To compare the number of loads containing native vs. non-native plant pollens within each season, we used Chi-square tests. We used a Chi-square test to determine whether the ratio of native to non-native flowers varied with season. Where appropriate, we applied the Sequential Bonferroni correction to correct for multiple testing of the same data. Tests that pass the correction are

indicated as [SB*]. We report averages as mean ± 1 standard error.

Results

Fluctuations in colony size

Over the course of our study, the mean colony size was 4683 ± 91 workers. On average, colonies were smallest during the *dormant* season (Fig. 1f; 4370 ± 257 workers) and largest during the *dry* season (Fig. 1f; 5131 ± 139 workers). We conducted a Univariate Repeated-Measures analysis to determine the effect of month on colony size. There is a significant effect of month on colony size ($F_{11,\ 59} = 3.04$, $p < 0.003$): August (*dry* season) corresponds to significantly larger colony sizes than March–April (beginning of the *growth* season, Tukey HSD test). Colony identity accounted for 18% of model variance. However, when grouped into the three seasons, our observation colonies exhibited relatively little fluctuation in size (Fig. 1f).

Effect of season on the level of waggle dancing

We decoded waggle dance performances from 235 bees that performed at least four consecutive waggle circuits from three colonies over 17 months (Fig. 1b, c). On average, we observed 11.88 ± 0.51 dance circuits per waggle dance performance. The mean overall foraging distance was estimated to be 1218 ± 74 m (median = 929 m). There is an overall effect of season on the number of waggle dancers per hour (Kruskal–Wallis test, $\chi^2_2 = 14.43$, $p < 0.0007$). There were significantly more waggle dancers during the *growth* season (Steel–Dwass $Z = 3.741$, $p = 0.0005$) and *dry* season (Steel–Dwass $Z = 2.581$, $p = 0.0266$) than during the *dormant* season (Fig. 1b).

Effect of season on foraging distances

On average, colonies recruited for closer pollen sources during the *dormant* season and for farther sources as the climate warmed. Bees flew, respectively, 1.6-fold and 2.7-fold greater foraging distances in the *growth* and *dry* seasons as compared to the *dormant* season. Mean foraging distances were 640.0 ± 618.6 m in the *dormant* season ($n = 28$), 1044.3 ± 93.36 m in the *growth* season ($n = 133$), and 1747.4 ± 125.2 m in the *dry* season ($n = 74$). There was a significant effect of season (Fig. 1c; ANOVA, $F_{2,\ 232} = 16.72$, $p < 0.0001$) on mean foraging distances. Colony accounted for only 1.1% of model variance. Foraging distance varied significantly between each pair of seasons (Tukey HSD, $\alpha = 0.05$, $Q = 2.36$). Monthly mean foraging distance significantly increased with monthly mean daily high temperature (ANOVA, $F_{1,\ 14} = 9.51$, $p = 0.008$, $R^2 = 0.51$).

Effect of season on foraging spatial distribution

The mean value of dispersion (R_D) across our study was 0.38 ± 0.18 ($n = 10$). This value of R_D is lower than 1, suggesting that bee-visited floral resources are not uniformly distributed in our study site. Seasonal measurements of R_D are significantly non-uniform in each season (Fig. 2b): *dormant* season ($R_D = 0.24$; $p < 0.001$), *growth* season ($R_D = 0.41$; $p < 0.001$), and *dry* season ($R_D = 0.60$; $p < 0.001$).

The highest monthly value of R_D occurred in June 2010 ($R_D = 0.58$). The lowest monthly value of R_D was measured in May 2010 ($R_D = 0.18$). We found no significant correlation between R_D and monthly mean daily high temperature ($F_{1,8} = 1.76$, $p = 0.22$). The spatial distributions of bee-visited floral patches appear to vary with season. Bees focused their recruitment dancing for pollen on few, clumped patches during the *dormant* season and performed increasingly wider searches for pollen with changes in season (Fig. 2b).

Effect of season on the number of flowering species

The number of native and non-native species in flower varied significantly with season ($\chi^2_1 = 31.34$, $p < 0.0001$; Fig. 1d). During the *growth* season, 631 species were in flower, 67% of which were native. During the *dormant* season, 184 species were in flower, 64% of which were native. During the *dry* season, 306 species were in flower, 54% of which were native. The number of native species in flower was significantly greater than that of non-native species during the *growth* season ($\chi^2_1 = 70.56$, $p < 0.0001$) and the *dormant* season ($\chi^2_1 = 13.59$, $p < 0.0002$). There was no significant difference in the number of native to non-native species in flower during the *dry* season ($\chi^2_1 = 1.307$, $p = 0.2529$).

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

We successfully amplified and sequenced DNA barcodes from 82% (217 out of 264) of corbicular pollen samples (these included samples from 29 bee dances that contained fewer than four dance circuits per dance). The mean sequence length was 715 ± 5.7 bp for *matK* ($n = 187$) and 526 ± 14.1 bp for *rbcLa* ($n = 30$). Over the three seasons, bees significantly shifted recruitment from native plants to non-native plants (Fig. 1e: LR $\chi^2_2 = 23.18$, $p < 0.0001$[SB*]). Bees collected pollen from five species (2.7% of 184

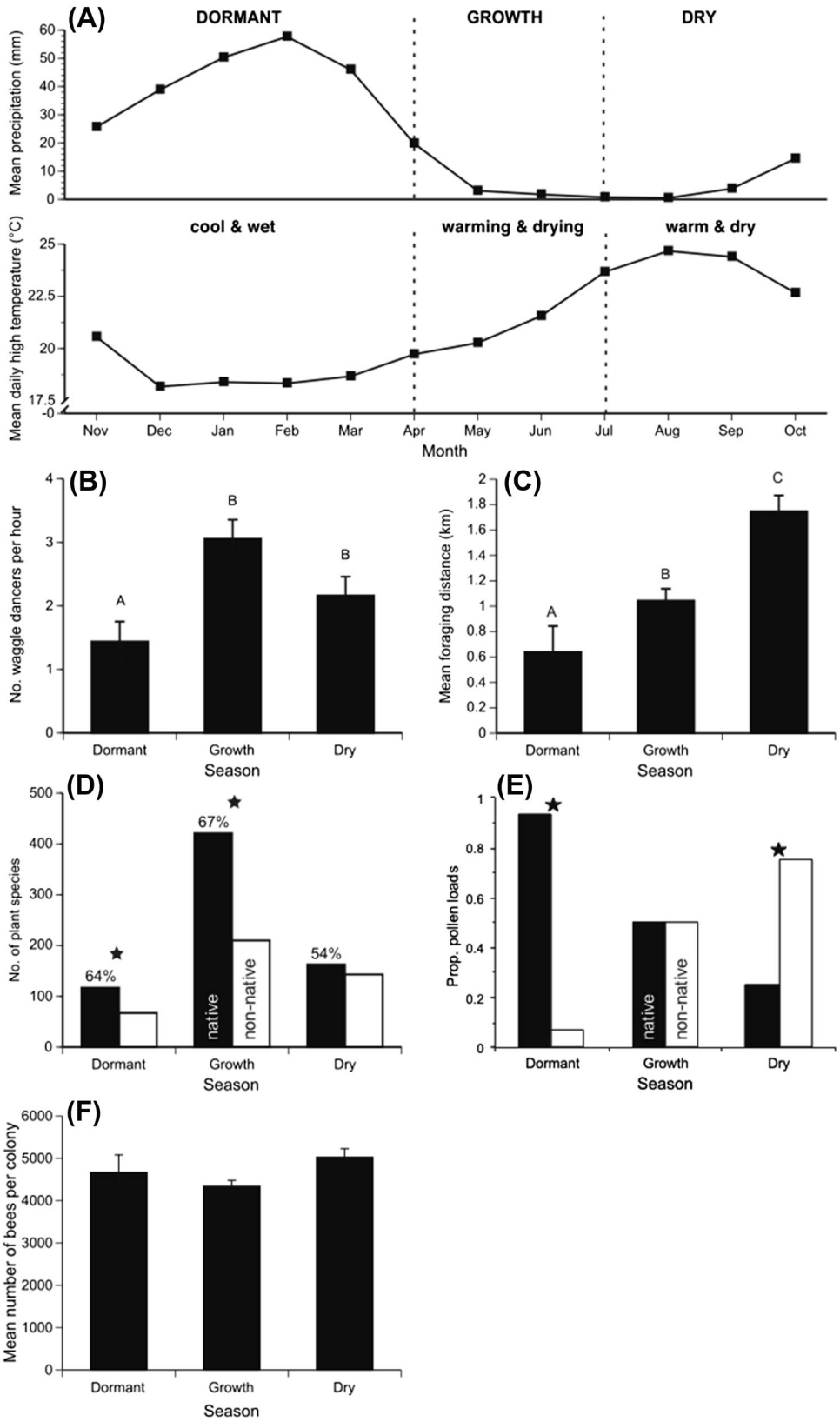

Fig. 1 Seasonal changes in waggle dancing, the number of plant species in bloom, pollen loads from native and non-native sources, and colony size. Standard *error bars* are shown. *Different letters* indicate significant differences. **a** Monthly change in mean daily high temperature and rainfall. **b** Seasonal shifts in the number of waggle dancers per hour. **c** Effects of season on mean foraging distances estimated from decoding waggle dances. **d** Effects of season on the total number of native and non-native plant species in flower. The percentage of native species in flower for a given season is shown above the *black bars*. *Stars* denote significant differences between the number of native and non-native plant species within a season. Data were collected from sources described in the methods. **e** Proportion of waggle dancer pollen loads collected from native or non-native sources for each season. *Stars* indicate significant differences within a season. **f** Seasonal changes in mean colony size. Data were pooled across all observation colonies. Data were pooled across colonies

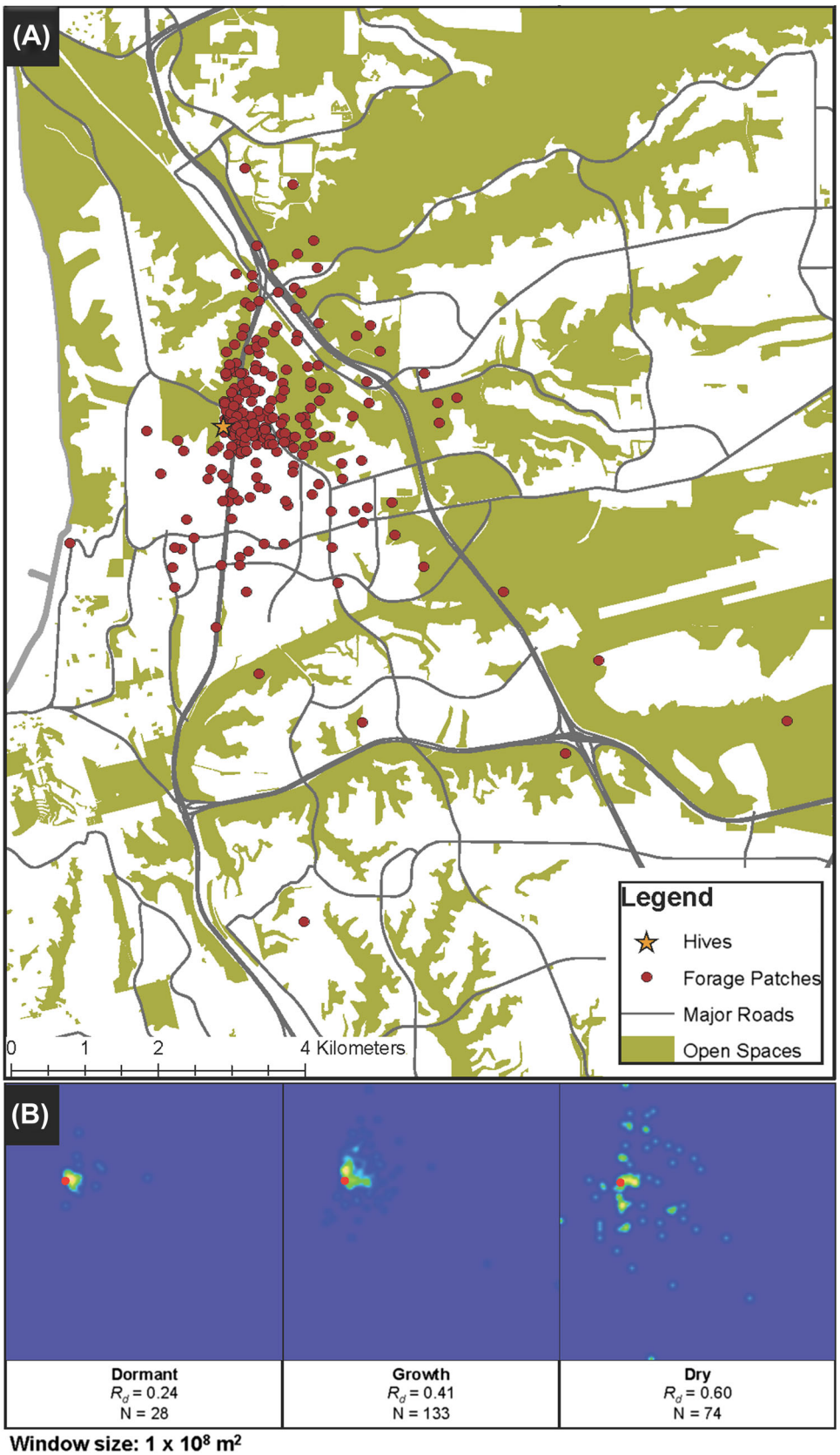

Fig. 2 Spatial distribution of bee-visited floral patches inferred from decoding waggle dances. **a** The location of all pollen foraging patches in the study area inferred from the waggle dance plotted onto a land-use map of the study site (LANDUSE-CURRENT, 2009; SANDAG, San Diego, California, USA). *White areas* indicate developed urban areas. *Green areas* denote open, undeveloped habitat fragments, which may include patches of native scrub and non-native grassland. 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*. **b** Density maps of bee-visited forage patches decoded from waggle dances for each season. *Warmer colors* denote a higher density of points at a 100 m^2 spatial resolution. Data were pooled from all colonies (location shown as *red dot*)

Table 1 Commonly collected plant taxa identified through DNA barcoding of bee-collected pollen loads

Season	Family	Probable identity	Common name	Habit	Status	Proportion of loads
Dormant	Anacardiaceae	*Rhus integrifolia*	Lemonade berry	Perennial shrub	Native	0.71
Growth	Anacardiaceae	*Toxicodendron diversilobum*	Poison oak	Perennial vine	Native	0.07
	Asteraceae	*Centaurea melitensis*	Maltese star thistle	Annual herb	Non-native	0.07
	Brassicaceae	*Brassica nigra*	Black mustard	Annual herb	Non-native	0.07
	Myrtaceae	*Eucalyptus* spp. (multiple species)	Gum tree	Tree	Non-native	0.12
	Polygonaceae	*Eriogonum fasciculatum*	California buckwheat	Perennial herb	Native	0.13
	Rosaceae	*Adenostoma fasciculatum*	Chamise	Perennial shrub	Native	0.12
	Rosaceae	*Heteromeles arbutifolia*	Toyon	Perennial shrub	Native	0.1
Dry	Asteraceae	*Baccharis pilularis*	Coyote bush	Perennial shrub	Native	0.11
	Myrtaceae	*Eucalyptus* spp. (multiple species)	Gum tree	Tree	Non-native	0.27
	Myrtaceae	*Melaleuca* spp.	Tea tree	Tree	Non-native	0.11

Taxa represented in this table were identified in >5% of all pollen loads collected within any given season. Data were pooled across all observation colonies

species) in flower during the *dormant* season. Native plants were highly important in the *dormant* season, representing 90% of pollen loads sampled during the *dormant* season (significantly more than from non-native plants, $\chi_1^2 = 13.76$, $p = 0.0002^{SB*}$). During the *growth* season, bees collected from 40 species (6.3% of 631 species) in flower during that time. However, in the *growth* season, bees collected pollen in exactly equal proportions from native and non-native plants ($\chi_1^2 = 0$, $p = 1.0$). Finally, in the *dry* season, bees collected pollen from 32 species (10% of 306 species) in flower during that time. During the *dry* season, non-native plants, primarily trees and shrubs, became the most important source (65%) of recruited-for pollen ($\chi_1^2 = 7.38$, $p = 0.006^{SB*}$). The proportion of species from on which bees foraged upon varied significantly with season ($\chi_1^2 = 1701$, $p < 0.0001$). In total, bees collected pollen from 35 families, 55 genera, and at least 60 species (Table S1). Based upon our analysis of herbarium records, flowering plants in this area consisted of 94 families, 410 genera, and 815 species.

Discussion

Our study provides new information on honey bee foraging by combining the measurement and analysis of four features of seasonal colony life: long-term observation of bee foraging distances as measured through recruitment dances and regular, weekly sampling, molecular identification of the plant species being recruited for, recruitment diet breadth, and an analysis of how this recruitment shifts seasonally for native and non-native plants. Colonies recruited for pollen from a wide variety of plant species (Table S1), but focused their recruitment efforts on a relatively smaller subset of species, even when many species are potentially available as forage such as during the *growing* season in the study habitat (Fig. 1d; Table 1). Seasonally, colonies acclimated their recruitment to the changing abundance of pollen resources and increase foraging distances (up to 2.7-fold) for pollen resources during periods of seasonal dearth. Temperature positively correlated with and accounted for 51% of variance in foraging distances (Fig S1). This temperature effect is likely due to the overall seasonal changes in pollen availability, not thermal constraints on flight. For honey bees, 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). However, at our site, daytime temperatures were rarely outside this range (Fig. 1a).

We also demonstrated, not surprisingly, a decrease in pollen recruitment during the season of floral dearth (*dormant* season, Fig. 1b). Our method of randomly selecting and focusing in on pollen dancers may have underestimated pollen dancing during seasons of floral abundance and overestimated pollen dancing during the *dormant* season. However, these effects would have made our test more conservative by reducing the measured seasonal differences in pollen waggle dancing.

Interestingly, non-native plants therefore provided an important source of colony protein, given that the colony recruited for these species, during the *dry* season. Colonies exploited pollen from native and non-native plants but seasonally changed their recruitment upon native plants such that non-natives became a more important part of their recruitment efforts in the *dry* season (65% of recruiter pollen loads) as compared to the *dormant* season (10% of pollen loads).

This usage of native and non-native plants changed spatially. Overall, pollen resources that colonies recruited

for were closest to the nest site and were most spatially clumped during the *dormant* season and became more distant and dispersed in subsequent seasons (Fig. 2b). Honey bees primarily recruited for pollen from native species localized in scrub fragments (which primarily host native species) during the cooler seasons and collected pollen from more widely dispersed, non-native species during warmer seasons of the year. The spatial distribution and species composition of floral pollen resources will vary in different landscapes. However, the pattern observed at our site—a *dormant* season with a low number of species in flower followed by period of *growth* with a high number of flowering native species (Fig. 1f), followed by a distinct *dry* season—is characteristic of the Mediterranean-like climate of our study site (Cowling et al. 1996; Ackerly 2009).

Effect of season on the spatial distribution of pollen sources

Our colonies recruited, on average and across all seasons, to pollen sources 1128 m away from the nest. This distance is within the range reported by Waddington et al. (1994) who decoded foraging distances of 534–1138 m in February (mean of 706 m for pollen foragers) in a similar southern California suburban habitat (Riverside, California).

The spatial distribution of exploited floral patches (Fig. 2b) was most clustered during the *dormant* season ($R_D = 0.24$), became more uniformly distributed during the *growth* season ($R_D = 0.41$), and was most dispersed in the *dry* season ($R_D = 0.60$). Because of low forager numbers in the *dormant* season (a normal feature of honey bee colony lifecycle, Seeley 1995), we were unable to formally test whether patchiness of resources varied with season. However, our measurements of R_D suggest that there are seasonal differences in how colonies allocate foragers over the landscape to forage for pollen (Fig. 2b). As such, our results corroborate those of previous studies and indicate that seasonally induced changes in the spatial clustering of foraging are a general, repeatable pattern in colony foraging behavior. This pattern is likely driven by seasonal changes in flowering plant availability (Fig. 1d).

Bees recruited to the most distant food sources during the *dry* season, corresponding to seasonal food dearth, as compared to the *growth* season (Fig. 1c). Other studies also show that foraging distances increase during periods of dearth or in response to mass flowering of distant, patchy resources. Beekman and Ratnieks (2000) found that foragers flew significantly farther in August than in May. Couvillon et al. (2014) observed significant differences in mean foraging distances between spring (493 m) and summer (2156 m) but found no significant difference in mean crop sugar content between seasons, suggesting that seasonal changes in the availability of nearby resources, not nectar sugar content, may drive changes in foraging distances.

Could increasing colony size, not seasonal change, account for increasing foraging distance? Colonies change size with season (Seeley 1995), and thus seasonal change and colony size are linked as part of the natural history of honey bee. However, seasonal changes in mean foraging distance (Fig. 1c) were far greater than the fluctuations in colony size. This suggests that seasonal changes in food availability, not changes in colony size, were a major driver of changes in foraging distance. Fluctuations in our colony sizes (Fig. 1f) were relatively minor, perhaps because the local climate is relatively moderate throughout the year (Fig. 1a), natural food sources were available year-round (Fig. 1d), and colonies were kept in a temperature-controlled observation room.

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

Donaldson-Matasci and Dornhaus (2012) suggested that waggle dancing benefits colonies in habitats with many flowering species by directing foraging efforts to only the most profitable resources. Our data support this hypothesis because honey bee colonies concentrated their pollen recruitment efforts on common species that provided abundant pollen (Fig. 1e; Table 1). Based upon herbarium records of available flowering species in the three seasons, we found recruitment diet breadths of 2.7, 6.3, and 10% in the *dormant*, *growth*, and *dry* seasons, respectively. This is far lower than the diet breadth reported by other studies: bees collected pollen from 30% of available plant species in a Texas coastal prairie (Baum et al. 2004) and 25% in Greece (Dimou and Thrasyvoulou 2007). It is possible that many of the floral species found in San Diego are not suitable for honey bees (i.e., are putatively described as wind-pollinated species), are not abundant, or both. However, it is more likely that honey bees only recruit for a small subset of the species with available pollen.

During the *dormant* season when mean foraging distances were relatively short, colonies recruited mainly for *Rhus integrifolia*, a native, winter-flowering shrub that is common in the study area. During the *growth* season, foraging distances increased and colonies recruited mainly for seven abundant and widely distributed spring-flowering native shrubs and invasive herbs (Table 1). During the *dry* season, we observed the greatest mean foraging distances, and colonies mainly recruited for *Baccharis pilularis,* an autumn-flowering native shrub common in coastal scrub habitats throughout southern California, and several non-native Eucalypt species. These species are often the only flowering plants available to bees during the drier, warmer parts of the year. Thus, introduced plant species likely

provided an important protein subsidy to honey bee colonies during the *dry* season, after many native species were no longer available.

To determine the impact of seasonality of colony use of available pollen resources, it would be ideal to use larger colonies that would enable researchers to capture colony behavior in a more realistic way. It is possible to build far larger observation colonies, although the standard two-dimensional design of such a colony offers a far larger surface area for cooling than would normally occur and even placement in a heated room would introduce an artificial subsidy to the colony. Perhaps the development of cameras with wide fields of view that could fit inside standard colonies would be helpful. However, our data suggest that non-native plants can be an important source of pollen to honey bee colonies in southern California. Whether this is also true in other habitats is an interesting question that calls for studies at multiple sites.

Using DNA barcodes to study plant–pollinator interactions

DNA barcoding can illuminate the vital ecosystem service provided by bee pollinators (Wilson et al. 2010) and help identify which plant species sustain bee populations. Researchers can identify pollen to genera or species using standard PCR and Sanger sequencing techniques, without specialist training or amassing a large library of pollen reference slides. Molecular records of plant species also provide growing and valuable open access data for other researchers and can be built with any plant tissue, not just pollen, which is only available at certain times of year.

The potential for honey bees as effective pollinators of native plants in disturbed habitats

The precise importance of honey bees as pollinators of native plants is disputed (Aizen et al. 2008; Potts et al. 2006; Kaiser-Bunbury and Memmott 2009). However, in fragmented environments where native pollinator species richness is lower than protected reserves (Hung et al. 2015) and isolation between fragments is high, the long-distance foraging capabilities of honey bees may compensate for the loss of native pollinators and connectivity between populations (Aguilar et al. 2006).

In our study, honey bees recruited for six species of native plants (54% of all plant species that bees mainly recruited for) over the course of a year. Overall, 71, 42, and 11% of dancers recruited for native plants in the *dormant*, *growth*, and *dry* seasons, respectively (Table 1). Honey bees also recruited to non-native plants, most of which are ornamental species (e.g., various Eucalypt species; Table 1), and two of which are invasive species that are common to the study area and flower during the *growth* season (*Centaurea melitensis* and *Brassica nigra*; Table 1). Despite this, only 14% of pollen loads assayed during the *growth* season were identified to these species. Considering the nested architecture of pollinator–pollinator interactions (Bascompte et al. 2003), the pollination services rendered by honey bees may be useful in the maintenance of important, locally abundant, common plant species, which serve as important food resources for both generalist and specialist pollinators and help stabilize plant–pollinator networks (Rohr et al. 2014). It would also be good to consider, in future studies, the effects of non-native honey bees and non-native plants upon native bees, native plant communities, and the stability of pollinator networks.

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