



RESEARCH PAPER

Pollen collection by honey bee hives in almond orchards indicate diverse diets

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Abstract

Almond is one of the world's most economically valuable crops and many varieties require cross pollination for optimal fruit set. For this reason, western honey bee (*Apis mellifera* L.) hives are often placed in almond orchards. However, little is known about the usage of almond and other pollen sources by individual hives during almond bloom. Here, we investigated the timing, identity and quantity of pollen collection associated with almond floral abundance and spatial location of individual hives by sampling 440 individual pollen tray samples and counting 45,072 pollen grains from 13,200 pollen pellets collected from 80 individual hives across the flowering season in Victoria, south-eastern Australia. A large proportion of hives collected non-almond pollen in addition to almond pollen (63/80 = 79%). The weight of almond pollen collected by the hives at each sampling time was positively related to the number of concurrently open almond flowers. However, non-almond pollen richness and abundance was not related to the number of almond flowers but had a positive relationship with the weight of almond pollen collected. There was no relationship between the distance among hives and identity of pollen collected. Yet, three plant families in the study area were found to account for a high percentage of the non-almond pollen collected, Euphorbiaceae, Fabaceae and Asteraceae. Understanding crop and non-crop pollen collection could inform honey bee diet needs and identify the plant species of importance to inform best practice bee management during almond flowering.

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Keywords: Pollen diversity; Mass flowering crop; Honey bee diet; Semi-natural vegetation

Introduction

Most almond (*Prunus dulcis* Mill.) crops rely on pollinators to produce marketable nuts (Cunningham et al., 2002).

As most almond varieties are self-incompatible, cross-pollination between at least two compatible varieties is necessary to obtain high yields (Ballester et al., 1998; Wirthensohn et al., 2011). Honey bees (*Apis mellifera* L.) are the most common managed pollinator used to provide pollination services for almond and several other high value crops (Breeze et al., 2011; Gallai et al., 2009; Klein et al., 2007; Southwick & Southwick Jr 1992). Their importance as managed

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pollinators is a result of their generalist flower-visiting behaviour across a large number of crops, social behaviour with high worker recruitment, colony availability throughout the year and ease of management and transport (Calderone, 2012; Olesen et al., 2010).

Honey bee individuals can exhibit different foraging behaviours due to age, experience and genetic characteristics, resulting in some individuals collecting more or less pollen/nectar than others (Hunt et al., 1995). As a highly eusocial species, flower visitation and pollen collection may also be guided by nutrient needs at the colony level (Hendriksma et al., 2019), colony strength (Chaand et al., 2017) or by the colony location in relation to non-crop habitat (Guzman et al., 2019). This means that although honey bees are generalists, they may alter their foraging behaviour in response to the availability or identity of floral resources surrounding a farm in which they are placed for crop pollination (Kwak & Jennersten 1991; Requier et al., 2015; Williams & Kremen, 2007). For example, when non-crop resources (weeds and wildflowers) are available near less attractive crop flower options, honey bees may switch to non-crop resources (Girard et al., 2012; Pettis et al., 2013). Although some studies have quantitatively evaluated the relationship between crop and non-crop floral resource collection during crop flowering (Fijen et al., 2019; Guzman et al., 2019; Woodcock et al., 2013), it is still unclear what governs resource collection by individual honey bee hives when colonies are inserted into mass flowering crops.

Pollen richness and abundance collected by honey bee colonies can vary over a given season, depending on floral resource availability and selection by honey bees (Decourtye et al., 2010; Odoux et al., 2012; Requier et al., 2015). Although pollen resources in mass-flowering crops may be abundant in a given season, monocultures lack pollen diversity and a lack of natural or semi-natural habitats can result in a lack of diversity of floral resources available overall (Decourtye et al., 2010; Rader et al., 2014). Given that a diverse and balanced diet of pollen nutrients is required to maximise brood production in honey bees (Brodschneider & Crailsheim 2010; Schmickl & Crailsheim 2004), the nutritional requirements of honey bees can be compromised in agriculturally intensive monocultures (Naug, 2009).

The goal of this study was to investigate the quantity and identity of almond and non-almond pollen collected by honey bees at different stages of the almond flowering season and the extent to which the spatial location of individual hives influences the identity of pollen collected. Specifically, we asked three research questions:

1. How much almond pollen do honey bees collect in relation to almond floral abundance and does the quantity collected vary at different stages of almond flowering?
2. What is the quantity and identity of non-almond pollen collected by individual honey bee hives in almond orchards?
3. To what extent is the non-almond pollen collected by honey bees related to the spatial location of the hives in the apiaries?

Materials and methods

Study site

The study was conducted in two large almond orchards, “Narcooyia” (2,375 hectares with 220 to 240 trees per hectare) and “Lake Powell” (1,200 hectares with 220 to 240 trees per hectare) near Lake Powell, Victoria, in south-eastern Australia (- 34.733747, 142.927534) in 2017 (Fig. 1). In our study area, each orchard was formed by several blocks of almond trees within a large orchard property, and each orchard had one apiary (i.e. a beekeeper’s stock of hives) distributed in several hive sites (i.e. a collection of those hives in a particular location). We collected data at three different sites, two in the Narcooyia apiary (NC02 and NC06, 20 hives from each site from a total of 96 hives); and one site in the Lake Powell apiary (LP20; 40 hives used for data collection from a total of 71 hives). The three hive sites sampled were at the edges of different almond blocks. The hives were grouped into clusters of 2 to 4 hives placed next to each other (<0.2 m) and the distance between each cluster was 2–2.5 m. Distance between blocks was 5–6 m and orchards were separated by the same distance as where one orchard’s block ended the other orchard’s block began. Non-almond floral resources were present within the orchard boundaries in a few scattered patches and some individual weeds along the almond tree rows. In addition, there were large areas of natural habitats on the boundaries on the north side of the orchards, adjacent to Lake Powell, Carpul Wildlife Reserve and the semi-natural vegetation along the Murray River. NC02 was the closest hive site to this area (0.7 km), while NC06 was set 1 km away and LP20 was set 2 km away (Fig. 1).

To identify the plant species present in semi-natural vegetations within our study region and land use types around the study sites, land use types were converted into land cover categories polygons modified from the Victorian Land Use Information System (Morse-McNabb et al., 2017), using QGIS version 3.12 and satellite imagery derived from Bing Aerial via the OpenLayers plugin <http://www.openlayers.org>. For each apiary site (NC02, NC06 and LP20), 1, 2.5, and 5 km radius buffers were then created, and these were intersected with plant observation records obtained from the *Atlas of Living Australia* (2022) and land cover polygons to derive plant species lists and percentage of land cover types at each scale (see Appendix A: Fig. 1 and Table 1 and 2).

Almond flower abundance over the season

Almond orchard bloom can vary depending on the temperature and variety but generally lasts for 2 to 4 weeks (Hill et al., 1985; Rattigan & Hill 1986). Almond flowers start to appear on the trees in late July to early September in

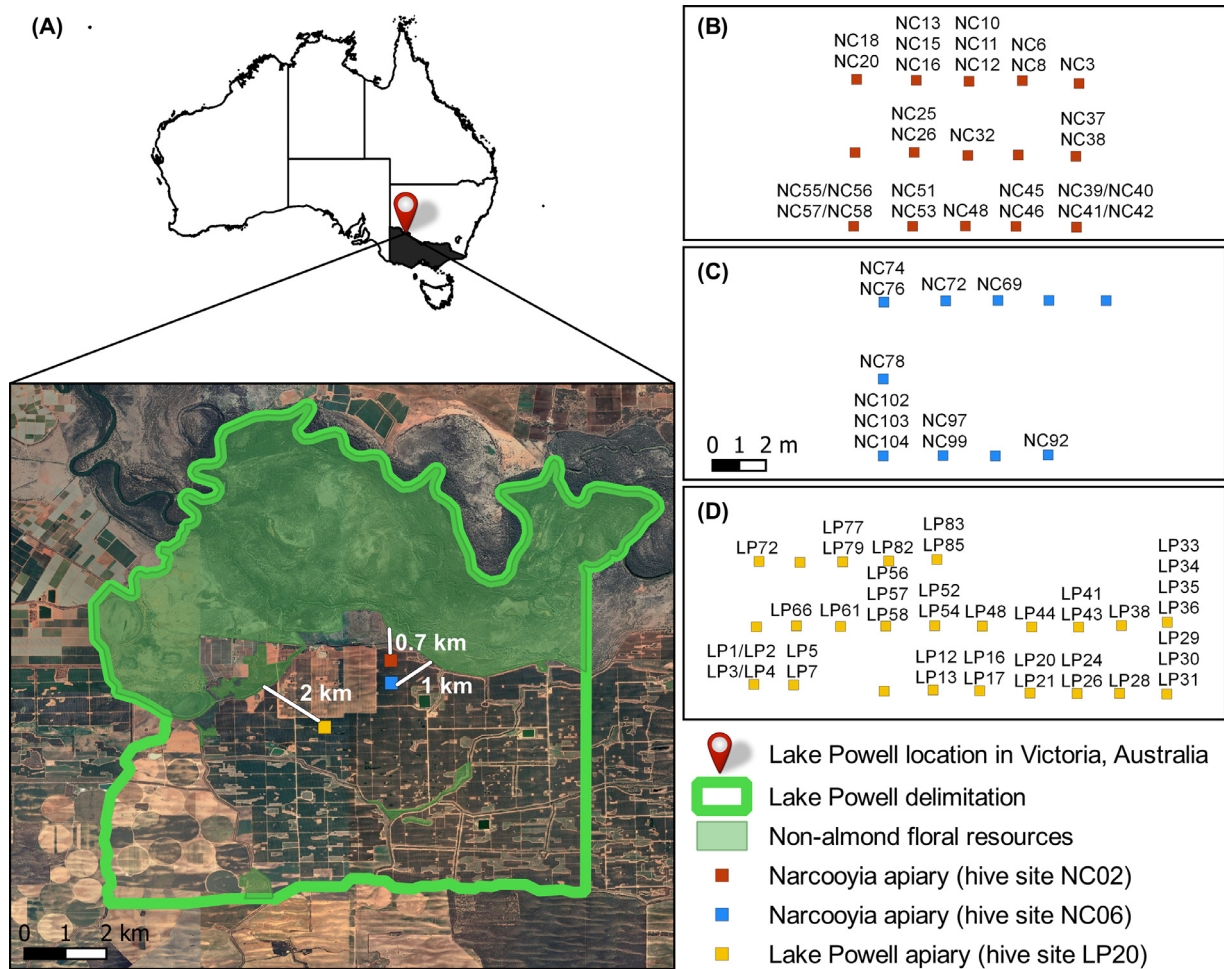


Fig. 1. (A) Location of Lake Powell in the State of Victoria, Australia. The green line indicates the boundaries. The large non-crop floral resources area is shaded in green, and the three boxes are the hive sites with approximately 2 km between them. At the Narcooyia apiary we took samples from two adjacent hive sites: (B) NC02 with 29 hives used for data collection and (C) NC06 with 11 hives used for data collection. (D) At the Lake Powell apiary (hive site LP20) we collected data from 40 hives. The boxes (B), (C) and (D) are a magnification of the hive positioning within the hive sites. Each red, blue and yellow box represents a cluster of hives (composed of two to four hives). In this figure, we only highlighted (writing the names) the hives that we used for pollen collection in each cluster. Distance between clusters of hives varied between 2–2.5 m and the nearest hive site to the non-almond floral resource area was NC02 located 1 km away.

Australia (Hill et al., 1985). In our study orchards, the almond plantings consisted of three almond varieties: Nonpareil, Carmel and Price. Early and late stages of orchard bloom normally have fewer available flowers as the Price variety flowers first, followed by the Nonpareil and then the Carmel. During our study year, the almond trees bloomed from 10 August to 3 September with the peak bloom around 21 August when the whole orchard was in bloom.

Almond buds and flowers were counted on the same day that pollen sampling occurred. Counts of opened flowers were conducted on tagged branches. A total of 60 branches were selected on 6 August 2017 including all the three varieties (Carmel, Nonpareil and Price), there was no loss due to seasonal damage. Bud and bloom counts were taken from tagged branches for a total of 11 sampling days, on alternate days (every 48 h) starting 12 August 2017 and finishing 1 September 2017.

Sample collection

Each ten-frame, Langstroth-type hive contained between 6.5 and 9 frames (with approximately 3,000 honey bees per frame) and were all managed and owned by the same commercial beekeeper. All the hives were placed into almond orchards 7 days before anticipated flowering. At each apiary, 48 h before the first pollen collection, we fitted Nuplas complete pollen traps[®] over the entrance of the hives in order to let them to acclimatise. After this initial 48 h, the tray of pollen pellets was emptied and fitted again, and we waited another 48 h with the pollen tray actively collecting pollen to remove the first pollen sample (on 12 August). Then we collected the pollen samples on alternate days (every 48 h) during bloom between 12 August and 1 September 2017, i.e. eleven sampling days. Sampling occurred in the mornings before temperatures rose above 13°C. To reduce stress due to

pollen being removed from the bees, hives were divided into two groups of forty (20 in each apiary). Hives in each group were matched based on their number of frames so that an equal amount of hives containing between 6.5 to 9 frames of bees were present in both groups. The first 7 days of pollen pellet collection (from 12 to 18 August) were taken from the first group of 40 hives (Group A) and the remaining 14 days of data collection (from 20 August to 1 September) were collected from the second group of 40 hives (Group B). A total of 440 individual pollen tray samples from 80 hives were collected across the flowering season.

Pollen analysis

After pollen pellets were collected from the pollen traps, samples were taken to the laboratory and divided into two sub-samples for each hive: almond pollen pellets and non-almond pollen pellets. We identified each pellet as either almond or non-almond pollen by colour and then weighed almond and non-almond pollen sub-samples. Sub-samples combined had a mean \pm SD weight of 15.55 g \pm 24.97 g. The samples were stored frozen until further processing and were dried in a dehydrator before acetolysis. This last step allowed us to remove any remaining water that could react with the acids.

For each sample from the hives, the sub-samples with non-almond pollen pellets were crushed, homogenized and 0.5 g were separated to perform acetolysis following the method outlined by Jones (2014). After acetolysis, for each sample, two slides were mounted swabbing the bottom of the tube with Fuchsin gelatine to stain the pollen grains and increase the contrast for identification. In order to standardize the counting and identification of species in each sample, we used a slide grid and counted pollen grains in transects. We chose the two transects by setting a "cross" counting the middle square (where the transects met) only once. In total, 45,072 non-almond pollen grains were counted under a microscope with a mean \pm SD of 112 \pm 66 pollen grains per slide. Non-almond pollen grains were identified to family level (67% of the pollen grains) or to genus level (33%) using a pollen reference collection, and existing identification keys (Erdtman, 1986; Moore & Webb 1978; Salgado-Labouriau, 1973).

As we distinguished the pollen grains of almond by colour prior to acetolysis, we conducted additional validation steps to ensure that the colour deemed to be almond pollen pellets contained pollen exclusively from almond flowers. To do this, we combined the sub-samples of almond pollen from different hives within a group and randomly chose 25 pollen pellets. We did this for each group, totalling 50 samples. These samples were processed and counted with the same methods as the non-almond sub-samples. For all pollen pellet samples assumed to be almond pollen, at least 98.5% of the pollen grains were exclusively almond (Appendix A: Table 3).

Statistical analysis

Quantity of almond pollen collected by honey bee hives at different stages of almond flowering season

To analyse the relationship between the quantity of almond pollen collected and the abundance of almond flowers we constructed a linear mixed effects model in the "lme4" package in R (Bates et al., 2014) taking the total almond pollen weight as response variable; almond floral abundance as a fixed factor, and collection day and apiary identity as random factors (Table S4). The models were fitted using the *lmer* function and the statistical significance was tested using a likelihood ratio test comparing the model with and without the fixed factor using the R package "car" (Fox & Weisberg 2018). Then, we performed a Kruskal-Wallis test for the comparison of the quantity (weight) of almond pollen collected at early, mid and late season. We used the data from the first 3 days (12, 14, and 16 of August), the 3 days in the middle of the season (20, 22 and 24 of August) and the last 3 days (28 and 30 of August, and 1st of September) of almond pollen collected between those three periods (n = 120 for each group). We then performed Dunn's test as a post hoc analysis to determine which pairs of groups differed significantly.

Quantity and identity of non-almond pollen collected by honey bee hives at different stages of almond flowering season

We constructed six different models (Appendix A: Table 4), with the total non-almond pollen abundance, richness, and weight as response variables; either almond floral abundance or almond pollen weight as fixed factors, and collection days and apiary identity as random factors. For these models we tested (i) non-almond pollen abundance as a function of almond floral abundance and almond pollen weight; (ii) non-almond pollen richness as a function of almond floral abundance and almond pollen weight; and (iii) non-almond pollen weight as a function of almond floral abundance and almond pollen weight, using linear mixed effects models with *lmer* function in the "lme4" package in R (Bates et al., 2014). The statistical significance was tested using a likelihood ratio test comparing the model with and without the fixed factor using *anova* function in the R package "car" (Fox & Weisberg 2018).

To understand the differences in the identity of pollen collected between individual hives, we built an interaction matrix for the hives that collected non-almond pollen where the rows represented the different hives and the columns the pollen morphotypes identified. A pollen transport network was built based on this matrix, and we calculated six indices at the node level (hives and morphotype pollen) that captured distinct topological properties of the nodes in the

network, focusing exclusively on hives: 1) degree, expressing the number of interaction partners (in this case, plant species) that each hive is linked to in the network; 2) species-level specialization d' , which quantifies how interaction frequencies of given hives deviate in relation to the availability of interaction partners (in this case, plant species) in the network, with higher values indicating higher specialization in determined resources (Blüthgen et al., 2006); 3) species strength (s) is the sum of the proportions of interactions performed by a given hive/pollen morphotype across all its hive/pollen morphotypes; 4) betweenness centrality (bc) quantifying to what extent a hive lies on the shortest path between other pairs of hives, thus indicating the importance of a hive as a network connector; 5) closeness centrality (cc), which quantifies the proximity of a hive to all other hives in the network (i.e. hives sharing a number of plant species with other hives). Species with high cc values can have a large influence on other hives and vice versa and are useful for identifying important hives (Jordán et al., 2006); 6) partner diversity calculated as the exponential Shannon diversity and interpreted as a measure of generality of interactions, it is a weighted version of degree, down-weighting rare interactions (Dormann, 2011). All species-level indices were quantified by the function *species level* () in bipartite package version 2.14 (Dormann et al., 2008) in R (R Development Core Team 2019). In order to understand how hive level indices are determined by almond floral abundance, we performed linear models including indices at the nodes level (hives) as response variables and almond floral abundance as fixed effects. The linear models were fitted using the *lm* function and tested using ANOVA likelihood test with *anova* function.

Lastly, we performed a Kruskal-Wallis test for the comparison of the quantity (weight) of non-almond pollen collected at different time periods of the flowering season. We used the data from the 3 different groups (early, mid and late season, $n = 120$ for each group). Post hoc comparisons (Dunn's test) were applied.

Spatial location of the hives and similarity in non-almond pollen collection

Finally, we used Procrustes analysis to evaluate the congruence between the abundance of pollen grains collected and the spatial location of each hive. We built two matrices, one for non-almond pollen grain abundance with the hives in the first column and the plant families in the first row, and a distance matrix with the latitude and longitude for the same hives. We did that for all the 63 hives in which we found non-almond pollen. For the Procrustes analysis between the two matrices, we first performed a Principal Component Analysis (PCA) based on Euclidean distances, and pollen abundance data was Hellinger-transformed prior to PCA (Legendre & Gallagher 2001). Procrustes analysis

uses uniform scaling (expansion or contraction) and rotation to minimize the sum of the squared residuals between two matrices, resulting in the statistic m^2 (Gower, 1975; Peres-Neto & Jackson 2001). This statistic describes how close the two data configurations match, i.e. the degree of congruence. To perform this analysis, we used the R package “vegan” (Oksanen, 2015) with the *procrustes* function. To evaluate the significance (non-randomness) of the observed m^2 statistic we used the Procrustes permutation test (*protest* function; 10000 permutations; Oksanen, 2015).

Results

Quantity of almond pollen collected by honey bees at different stages of almond flowering season

The weight of almond pollen collected by honey bees from a single hive had a positive relationship with number of almond flowers at the time of pollen sampling ($\chi^2_{1, 440} = 50.40$, $P < 0.0001$, Fig. 2A). Moreover, the weight of almond pollen collected by the hives significantly varied throughout the season ($\chi^2_{2, 360} = 118.02$, $P < 0.0001$, Fig. 3A). For example, 70.44% of the pollen weight was collected in the first three days of data collection (early-flowering); 24.03% of the pollen was collected in the middle three days of the sampling period (mid-flowering) and 5.51% of the pollen was collected in the last three days of the sampling period (late-flowering; $P < 0.0001$ for all comparisons). A greater number of flowers blooming at the beginning of the season (see Appendix A: Fig. 2), most likely explains why almond pollen collection was greater in this period.

Quantity and identity of non-almond pollen collected by honey bees at different stages of almond flowering season

In contrast to the weight of almond pollen, the weight of non-almond pollen collected by honey bees was not related to the number of almond flowers blooming (see Appendix A: Fig. 3A) but had a positive relationship to the weight of almond pollen collected during the flowering season ($\chi^2_{1, 440} = 21.02$, $P < 0.0001$, Fig. 2B). We found a total of 18 morphospecies distributed in 9 families (see Appendix A: Fig. 4). The abundance and richness of these morphospecies also had a positive relationship with the weight of almond pollen collected ($\chi^2_{1, 440} = 9.24$, $P = 0.002$; $\chi^2_{1, 440} = 5.88$, $P = 0.01$, respectively), however, there was a very low effect in both models (Fig. 2C, D). Abundance and richness of non-almond pollen was not related to almond floral abundance ($\chi^2_{1, 440} = 0.32$, $P = 0.56$; $\chi^2_{1, 440} = 0.28$, $P = 0.59$, respectively, see Appendix A: Fig. 3B and C).

From the 80 hives analysed in this study, we found non-almond pollen grains in 63 hives (79%), but the collection of

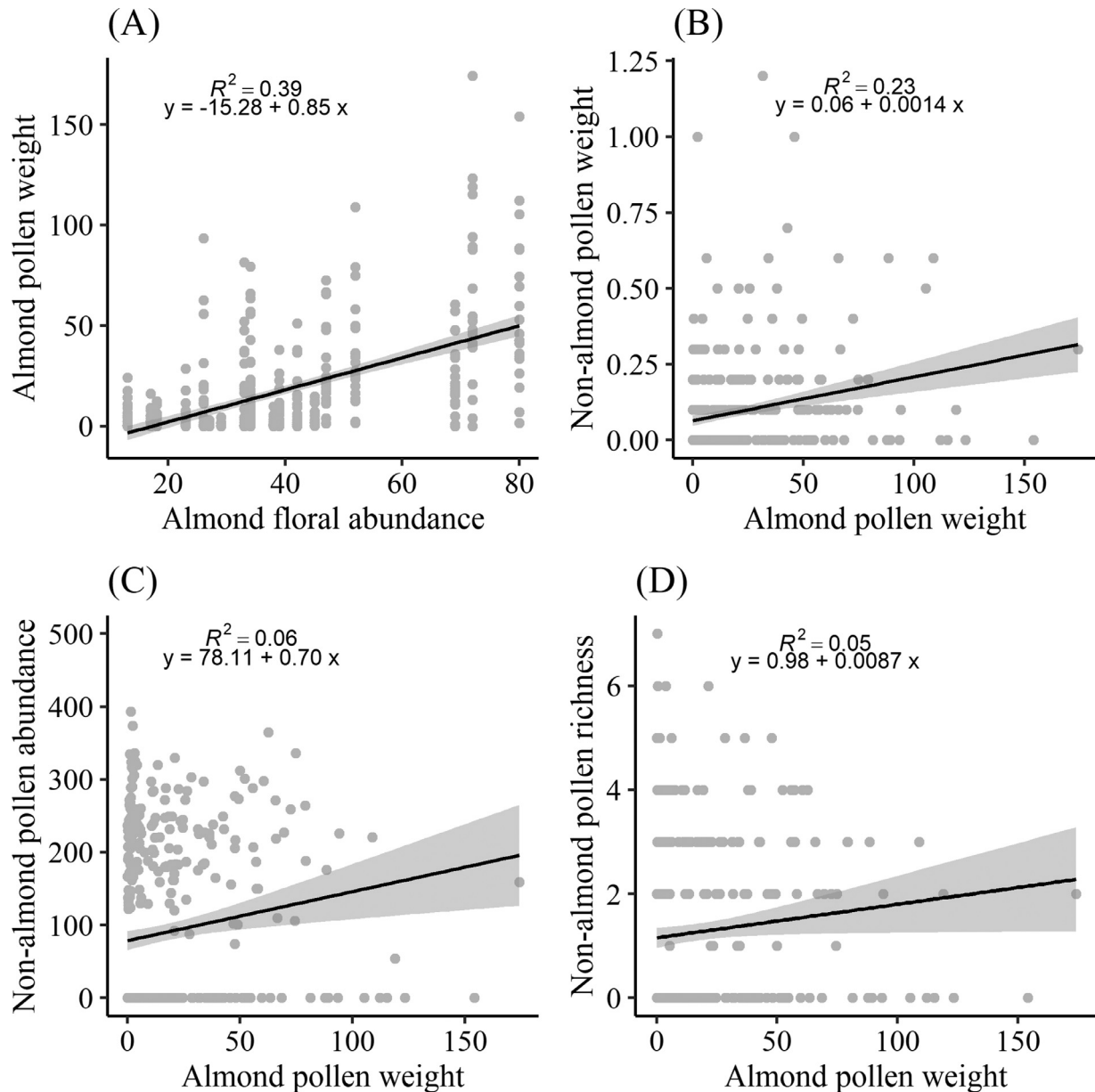


Fig. 2. (A) The estimated weight of almond pollen collected by honey bees in relation to the almond flower abundance ($\chi^2 = 50.40$, $P < 0.0001$). (B) The weight of non-almond pollen according to the weight of almond pollen grains ($\chi^2 = 21.02$, $P < 0.0001$), and (C) abundance and (D) richness of non-almond pollen collected by honey bees according to the weight of almond pollen ($\chi^2 = 9.24$, $P = 0.002$; $\chi^2 = 5.88$, $P = 0.01$, respectively). The almond floral abundance values represent the mean number of almond flowers per branch. Each dot represents a value ($n=440$) originated from individual pollen tray samples collected from each hive across the flowering season. The regression line shows the relationship between the variables and the grey shaded area around each regression line are the confidence intervals (95 %). The values annotated in each figure represent the R square and regression line equation.

non-almond pollen was relatively low for most hives overall (0.03% - 17.94%, see details at hive level in Appendix A: Fig. 5, 6, 7, 8, 9, 10 and 11). For example, 52.29% of the non-almond pollen was collected in the early-flowering stage, 18.96% during mid-flowering and 28.75% during late-flowering (Fig. 3B). The collection of non-almond pollen differed between early- and mid-flowering stages, and early- and late-flowering stages of the season ($P < 0.0001$ for both), but not between the mid- and late-flowering stages ($P = 0.33$).

None of the calculated indices at the node level were significantly correlated with the number of almond flowers throughout the season (see Appendix A: Fig. 12). Although there was no correlation with almond flower abundance, some bee hives had a greater number of interactions with a greater number of plant species, for example, while hive LP29 had a degree = 1, the degree for hive LP58 was 10. The other indices like specialization d' , species strength, and partner diversity all had very similar values (please see

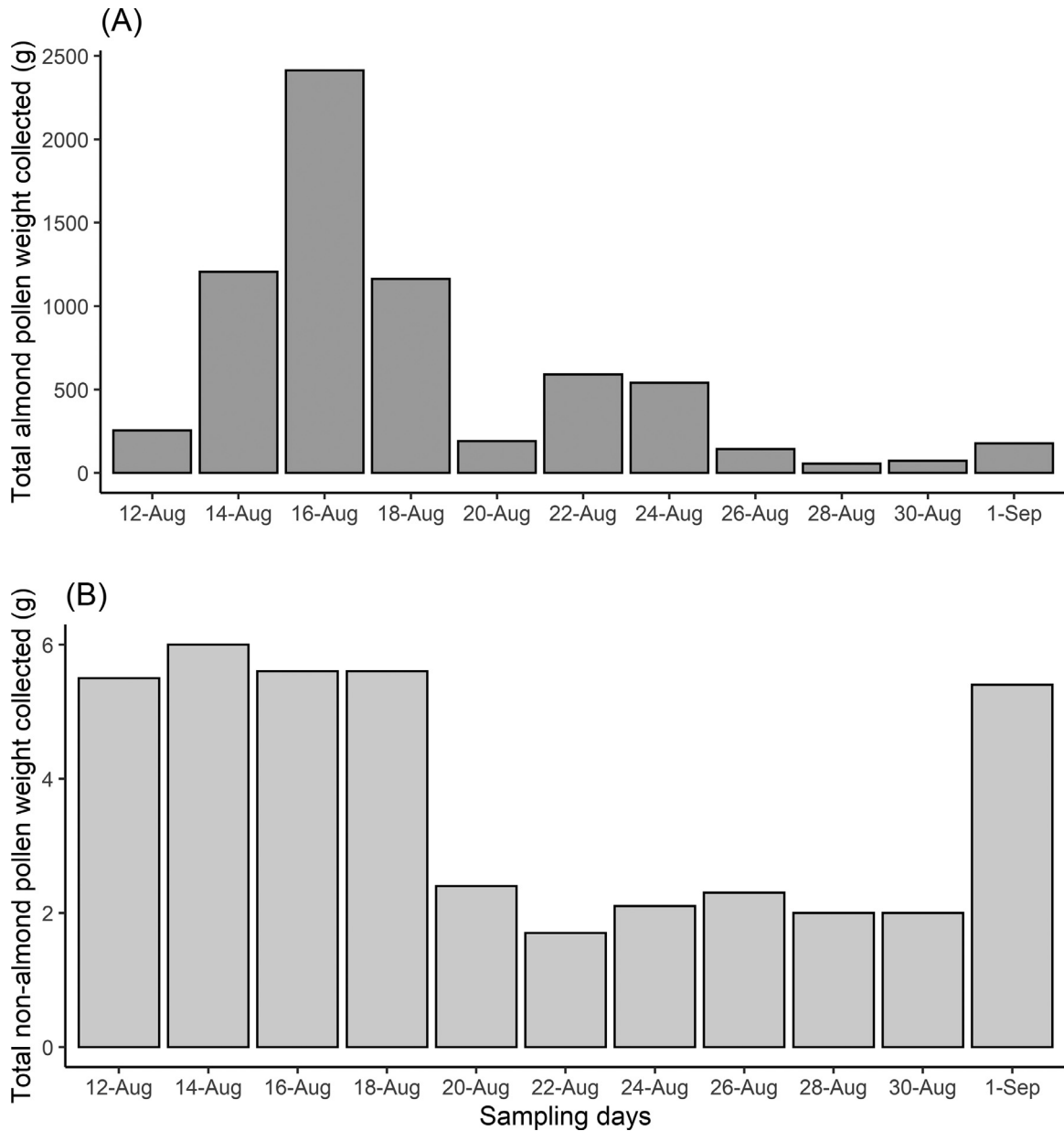


Fig. 3. The average weight of (A) almond and (B) non-almond pollen collected by the 40 hives on each sampling day throughout the almond flowering season.

Appendix A: Table 5 for all values per hive) for all the hives, but betweenness centrality and closeness centrality showed almost the same value for all the hives, indicating that hives had a similar impact and importance in connecting different parts of the network.

Some plant species in the network (Fig. 4) had a greater degree value indicating the number of connections they established with the hives. Euphorbiaceae sp1 and *Acacia* sp2 had a degree = 61, and consequently a higher species strength value suggesting that hives depend more on this species than Proteaceae sp2 which had a degree = 1, for example. These two pollen morphotypes were used by 96% of the hives that collected non-almond pollen (Appendix A:

Table 6). Additionally, a pollen type from the Asteraceae family (*Taraxacum* sp.) was linked to 63% of the hives with a higher value of species strength as well, and this last species is considered a weed in the study area.

Hive location and similarity in non-almond pollen collection

The distance among hives was not related to non-almond resource collection, demonstrated by a lack of significant correlation between the two ordinations ($r = 0.14$,

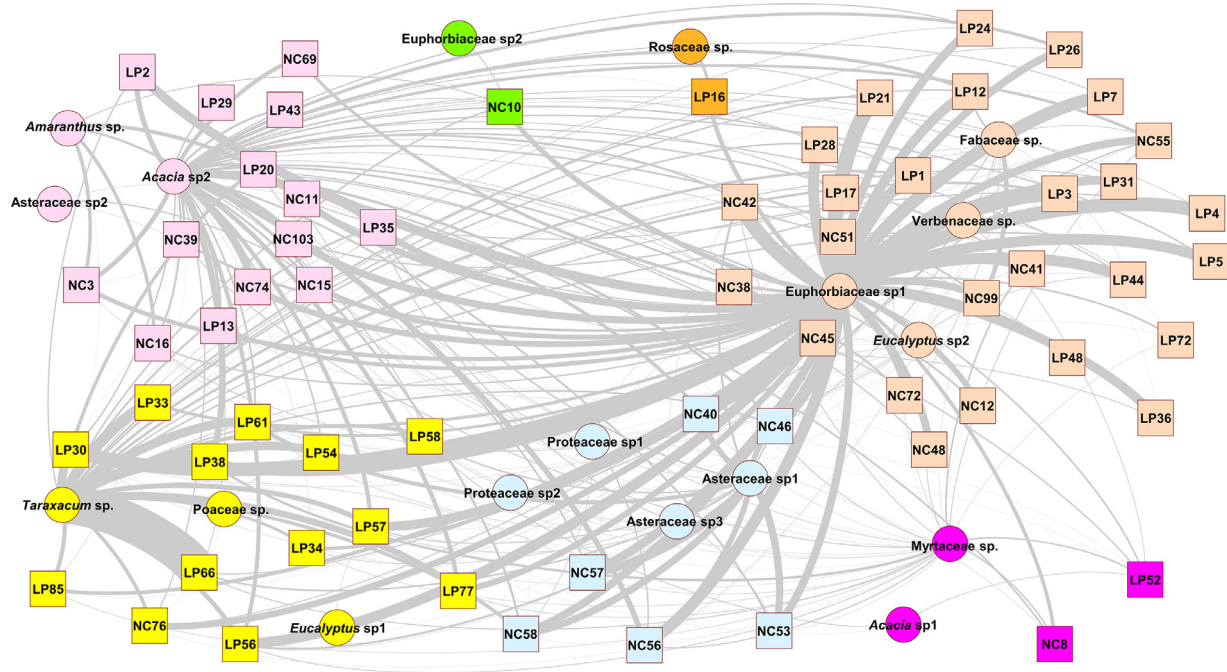


Fig. 4. Pollen transport network based on interactions between plant species (circles) and hives (boxes). The thickness of lines (grey) represents the abundance of non-almond pollen grains collected from each plant species by each hive. Plant species and hives formed seven different modules indicated by each colour. Plants and hives within the same cluster have a dense connection between them, but a sparse connection between nodes in different clusters. Hives in the same cluster not necessarily are close to each other in the study area, i.e., hives NC40, NC46, NC58, NC57, NC56 and NC53, from the same hive site, were strongly associated with the four plant species coloured in the same colour. However, the purple colour shows that hives of two different apiaries LP52 and NC8 were the ones that established strong interactions with the species *Myrtaceae* sp and *Acacia* sp1.

$m^2 = 0.97$, $P = 0.48$, see Appendix A: Fig. 12). This suggests that even hives close to each other did not collect the same pollen types, so it is likely that other factors are influencing pollen collection over time.

Discussion

Almond pollen collection by managed honey bee hives in flowering almond orchards was positively related to the number of almond flowers blooming and varied across the flowering season. This suggests that the bees are responding to almond floral abundance over the course of the short flowering season. This tight relationship is very likely due to the large number of almond flowers being offered, much higher than any other in the 500 m around the beehives. It remains to be seen whether bees also disproportionately look for this pollen due to its nutritional qualities (Loper & Berdel 1980; Todd & Bretherick 1942).

While several studies have shown considerable temporal variation in honey bee pollen harvest over a given season (Decourtye et al., 2010; Proesmans et al., 2019; Wratten et al., 2012), our study shows that most hives collected a range of different pollen types and almond pollen was the most abundant during the season. We found 21.25% ($n = 17$) of the hives in our study exclusively collected almond pollen

throughout the season. Of the remaining hives ($n = 63$) which collected both almond and non-almond pollen, at least 82% of the total pollen brought to the hives was from almond flowers, but these results could not be explained by their relative spatial position in relation to each other. Even though the collection of non-almond pollen was relatively low for most hives overall, the abundance of non-almond pollen found in hives across the flowering season was higher at early- and late-flowering.

The availability of many different types of pollen is thought to support a balanced and nutritional diet for honey bees (Di Pasquale et al., 2013; Schmidt, 1984), even in a mass-flowering environment with an abundance of the same crop as a resource (Alaux et al., 2010; Requier et al., 2015). As single species monocultures may compromise the nutritional needs of bees potentially affecting bee longevity and colony health (Branchiccela et al., 2019; Brodschneider & Crailsheim 2010; Di Pasquale et al., 2016; Mattila & Otis 2006), a diversity of pollen can result in greater colony strength by way of decreasing its nutritional stress (Filipiak et al., 2017) and increasing resistance against the effects of parasites (Huang, 2012).

Some hives interacted with a greater number of plant species than other hives as illustrated in the network, but they all performed a very similar role connecting different parts of the network. The connections established with plant

species could be related to the positioning of these hives in the study site, as some were closer to semi-natural areas than others (i.e. 0.7 km and 1 km to semi-natural habitat for NC02 and NC06 and 2 km for LP20) and proximity of hives to non-almond floral resources could lead to a greater number of interactions due to colony sharing information (Seeley & Visscher 1988). However, LP20, was the greatest distance from semi-natural areas, yet had a greater number of hives collecting non-almond pollen (36), followed by NC02 (22) and NC06 (6). Interestingly, the number of plant species recorded in the area was also greater around LP20 within all the different buffer sizes (1 km = 1 species, 2.5 km = 136 species, and 5 km = 378 species) comparing to NC02 and NC06 (1 km = 1 and 0, 2.5 km = 118 and 55, 5 km = 314 and 306, respectively). Therefore, despite other studies reporting the relationship between crop pollen collection and distance between crop and hives (Cunningham et al., 2016), greater research is needed at a greater number of sites to test this.

There was no correlation between the distance among hives and the pollen resources recorded at each hive. Therefore, hives in close proximity may forage for different resources depending on their needs. However, betweenness and closeness centrality presented very similar values for all hives, suggesting that they are doing similar things within the network. In the network, some hives from the three hive sites were connected to the same nodes, which means that the distance of 0.7 km–2 km to the non-crop vegetation may not be a limiting factor. Furthermore, as per optimal foraging theory, animals normally make decisions to minimize energy expenditure and maximize the intake of resources (MacArthur & Pianka 1966). Yet, foraging decisions in social animals can be more complicated as group benefits are an important consideration (Kay, 2002) and when analysing at colony level, these can vary from individual benefits (Hendriksma et al., 2019). Therefore, while our study was not designed to test the effect of distance to non-crop vegetation nor nutritional intakes, the position of the hives in relation to non-crop floral resource may be important and some hives may vary in their nutritional needs over time (Donkersley et al., 2017), resulting in pollen collections from different plant species.

We found that one of the most collected pollen morphotypes, like *Taxaracum* sp., is considered to be a weed in the study area yet was of significant importance to bees, being the main supplier of non-almond resources for hives such as “LP66”. Several native plant species found in our study were also regularly used as a pollen source for the bees, such as *Acacia* species and morphotypes of the Euphorbiaceae family. This could be related both to the abundance of these species during the almond blooming season and/or their high nutritional value (Frias et al., 2016) as semi-natural areas, species-rich grasslands and hedgerows are known to offer a valuable resource despite being less abundant than a mass-flowering crop (Croxton et al., 2002; Öckinger & Smith 2006).

Our study limitations highlight issues that require further research. First, a greater number of apiary sites at different

distances to non-almond floral resource are needed to test the premise that the resource collected by the hives is being impacted by the distance to the non-almond floral resource area. Second, this study would have benefited from additional data on the plant community in the study area, including plant community composition, spatial location and nutritional benefit to bees. This may help to understand the mechanisms underlying visitation and/or collection of pollen from particular plant species. Finally, higher resolution data on pollen species identity, and honey bee hive health metrics such as hive weight and reproduction metrics, would inform potential trade-offs among the factors impacting individual, colony and apiary scale pollen collection.

Conclusions

Non-crop floral resources are collected by honey bees even during the bloom of a highly attractive crop. Some non-almond floral resources were used by a greater number of hives as shown in the pollen-transport network. However, future research is needed to determine the relationship between non-crop floral resources being collected, the distance between hives and non-crop vegetation, and colony health. Honey bee hive placement for crop pollination should take into consideration the presence of non-crop vegetation surrounding the orchard as bees are using alternative floral resources. This may be an important factor to support honey bee diet needs and their performance as crop pollinating agents.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.baae.2022.07.006](https://doi.org/10.1016/j.baae.2022.07.006) and on Figshare at [doi:10.6084/m9.figshare.20235636](https://doi.org/10.6084/m9.figshare.20235636)

References

- Alaux, C., Ducloz, F., Crauser, D., & Le Conte, Y. (2010). Diet effects on honeybee immunocompetence. *Biology Letters*, *6*(4), 562–565.
- Atlas of Living Australia (2022). Occurrence download at <https://biocache.ala.org.au/occurrences/search?q=qid:1645675808513> Accessed on 24 February 2022.
- Ballester, J., Bošković, R., Batlle, I., Arús, P., Vargas, F., & de Vicente, M. C. (1998). Location of the self-incompatibility gene on the almond linkage map. *Plant Breeding*, *117*(1), 69–72. doi:10.1111/j.1439-0523.1998.tb01450.x.
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2014). Fitting linear mixed-effects models using lme4. *ArXiv Preprint ArXiv:1406.5823*.
- Blüthgen, N., Menzel, F., & Blüthgen, N. (2006). Measuring specialization in species interaction networks. *BMC Ecology*, *6*(1), 1–12.
- Branchiccela, B., Castelli, L., Corona, M., Díaz-Cetti, S., Invernizzi, C., de la Escalera, G. M., Mendoza, Y., Santos, E., Silva, C., & Zunino, P. (2019). Impact of nutritional stress on the honeybee colony health. *Scientific Reports*, *9*(1), 1–11.
- Breeze, T. D., Bailey, A. P., Balcombe, K. G., & Potts, S. G. (2011). Pollination services in the UK: How important are honeybees? *Agriculture, Ecosystems & Environment*, *142*(3–4), 137–143.
- Brodshneider, R., & Crailsheim, K. (2010). Nutrition and health in honey bees. *Apidologie*, *41*(3), 278–294.
- Calderone, N. W. (2012). Insect pollinated crops, insect pollinators and US agriculture: Trend analysis of aggregate data for the period 1992–2009. *PLoS One*, *7*(5), e37235.
- Chaand, D., Sharma, D., Ganai, S. A., Norboo, T., & Sharma, S. (2017). Effect of colony strength on colony build up and foraging activity of *Apis mellifera* L. *Journal of Entomology and Zoology Studies*, *5*(6), 1369–1373.
- Croxton, P. J., Carvell, C., Mountford, J. O., & Sparks, T. H. (2002). A comparison of green lanes and field margins as bumblebee habitat in an arable landscape. *Biological Conservation*, *107*(3), 365–374. doi:10.1016/S0006-3207(02)00074-5.
- Cunningham, S. A., FitzGibbon, F., & Heard, T. A. (2002). The future of pollinators for Australian agriculture. *Australian Journal of Agricultural Research*, *53*(8), 893–900.
- Cunningham, S. A., Fournier, A., Neave, M. J., & Le Feuvre, D. (2016). Improving spatial arrangement of honeybee colonies to avoid pollination shortfall and depressed fruit set. *Journal of Applied Ecology*, *53*(2), 350–359.
- Decourtye, A., Mader, E., & Desneux, N. (2010). Landscape enhancement of floral resources for honey bees in agro-ecosystems. *Apidologie*, *41*(3), 264–277.
- Di Pasquale, G., Alaux, C., Le Conte, Y., Odoux, J.-F., Pioz, M., Vaissière, B. E., Belzunces, L. P., & Decourtye, A. (2016). Variations in the availability of pollen resources affect honey bee health. *PLoS One*, *11*(9), e0162818.
- Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L. P., Decourtye, A., Kretzschmar, A., Suchail, S., Brunet, J.-L., & Alaux, C. (2013). Influence of pollen nutrition on honey bee health: Do pollen quality and diversity matter? *PLoS One*, *8*(8), e72016.
- Donkersley, P., Rhodes, G., Pickup, R. W., Jones, K. C., Power, E. F., Wright, G. A., & Wilson, K. (2017). Nutritional composition of honey bee food stores vary with floral composition. *Oecologia*, *185*(4), 749–761. doi:10.1007/s00442-017-3968-3.
- Dormann, C. F. (2011). How to be a specialist? Quantifying specialisation in pollination networks. *Network Biology*, *1*(1), 1–20.
- Dormann, C. F., Gruber, B., & Fründ, J. (2008). Introducing the bipartite package: Analysing ecological networks. *Interaction*, *1* (0.2413793).
- Erdtman, G. (1986). *Pollen morphology and plant taxonomy: Angiosperms*. Brill Archive Vol. 1.
- Fijen, T. P. M., Scheper, J. A., Boekelo, B., Raemakers, I., & Kleijn, D. (2019). Effects of landscape complexity on pollinators are moderated by pollinators' association with mass-flowering crops. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1900) 20190387. doi:10.1098/rspb.2019.0387.
- Filipiak, M., Kuszewska, K., Asselman, M., Denisow, B., Stawiarz, E., Woyciechowski, M., & Weiner, J. (2017). Ecological stoichiometry of the honeybee: Pollen diversity and adequate species composition are needed to mitigate limitations imposed on the growth and development of bees by pollen quality. *PLoS One*, *12*(8), e0183236.
- Fox, J., & Weisberg, S. (2018). *An R companion to applied regression*. Sage publications.
- Frias, B. E. D., Barbosa, C. D., & Lourenço, A. P. (2016). Pollen nutrition in honey bees (*Apis mellifera*): Impact on adult health. *Apidologie*, *47*(1), 15–25. doi:10.1007/s13592-015-0373-y.
- Gallai, N., Salles, J.-M., Settele, J., & Vaissière, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics*, *68*(3), 810–821.
- Girard, M., Chagnon, M., & Fournier, V. (2012). Pollen diversity collected by honey bees in the vicinity of *Vaccinium* spp. Crops and its importance for colony development. *Botany*, *90*(7), 545–555.
- Gower, J. C. (1975). Generalized procrustes analysis. *Psychometrika*, *40*(1), 33–51. doi:10.1007/BF02291478.
- Guzman, A., Gaines-Day, H. R., Lois, A. N., Steffan, S. A., Brunet, J., Zalapa, J., & Guédot, C. (2019). Surrounding landscape and spatial arrangement of honey bee hives affect pollen foraging and yield in cranberry. *Agriculture, Ecosystems & Environment*, *286*, 106624.
- Hendriksma, H. P., Toth, A. L., & Shafir, S. (2019). Individual and colony level foraging decisions of bumble bees and honey bees in relation to balancing of nutrient needs. *Frontiers in Ecology and Evolution*, *7*, 177.
- Hill, S. J., Stephenson, D. W., & Taylor, B. K. (1985). Almond pollination studies: Pollen production and viability, flower emergence and cross-pollination tests. *Australian Journal of Experimental Agriculture*, *25*(3), 697–704.
- Huang, Z. (2012). Pollen nutrition affects honey bee stress resistance. *Terrestrial Arthropod Reviews*, *5*(2), 175–189.
- Hunt, G. J., Page, R. E., Fondrk, M. K., & Dullum, C. J. (1995). Major quantitative trait loci affecting honey bee foraging behaviour. *Genetics*, *141*(4), 1537–1545.
- Jones, G. D. (2014). Pollen analyses for pollination research, acetolysis. *Journal of Pollination Ecology*, *13*(21), 203–217.
- Jordán, F., Liu, W., & Davis, A. J. (2006). Topological keystone species: Measures of positional importance in food webs. *Oikos*, *112*(3), 535–546.
- Kay, A. (2002). Applying optimal foraging theory to assess nutrient availability ratios for ants. *Ecology*, *83*(7), 1935–1944.

- Klein, A.-M., Vaissiere, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, 274(1608), 303–313.
- Kwak, M. M., & Jennersten, O. (1991). Bumblebee visitation and seedset in *Melampyrum pratense* and *Viscaria vulgaris*: Heterospecific pollen and pollen limitation. *Oecologia*, 86(1), 99–104.
- Legendre, P., & Gallagher, E. D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia*, 129(2), 271–280. doi:10.1007/s004420100716.
- Loper, G. M., & Berdel, R. L. (1980). The effects of nine pollen diets on broodrearing of honeybees. *Apidologie*, 11(4), 351–359.
- MacArthur, R. H., & Pianka, E. R. (1966). On optimal use of a patchy environment. *The American Naturalist*, 100(916), 603–609.
- Mattila, H. R., & Otis, G. W. (2006). The effects of pollen availability during larval development on the behaviour and physiology of spring-reared honey bee workers. *Apidologie*, 37(5), 533–546.
- Moore, P. D., & Webb, J. A. (1978). *An illustrated guide to pollen analysis*.
- Morse-McNabb, E., Lewis, H., Sheffield, K., Robson, S., Hopley, J., & Clark, R. (2017). Victorian land use information system 2016 [Vector, Tabular]. Victoria: Department of Economic Development. doi:10.4226/92/590ABBE6EA3F1.
- Naug, D. (2009). Nutritional stress due to habitat loss may explain recent honeybee colony collapses. *Biological Conservation*, 142(10), 2369–2372.
- Öckinger, E., & Smith, H. G. (2006). Semi-natural grasslands as population sources for pollinating insects in agricultural landscapes: Population sources for pollinators. *Journal of Applied Ecology*, 44(1), 50–59. doi:10.1111/j.1365-2664.2006.01250.x.
- Odoux, J.-F., Feuille, D., Aupinel, P., Loublier, Y., Tasei, J.-N., & Mateescu, C. (2012). Territorial biodiversity and consequences on physico-chemical characteristics of pollen collected by honey bee colonies. *Apidologie*, 43(5), 561–575.
- Oksanen, J. (2015). *Vegan: An introduction to ordination*.
- Olesen, J. M., Dupont, Y. L., O’Gorman, E., Ings, T. C., Layer, K., Melian, C. J., Trøjelsgaard, K., Pichler, D. E., Rasmussen, C., & Woodward, G. (2010). From Broadstone to Zackenberg: Space, time and hierarchies in ecological networks. *Advances in Ecological Research*, 42, 1–69.
- Peres-Neto, P. R., & Jackson, D. A. (2001). How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. *Oecologia*, 129(2), 169–178. doi:10.1007/s004420100720.
- Pettis, J. S., Lichtenberg, E. M., Andree, M., Stitzinger, J., Rose, R., & Vanengelsdorp, D. (2013). Crop pollination exposes honey bees to pesticides which alters their susceptibility to the gut pathogen *Nosema ceranae*. *PLoS One*, 8(7), e70182.
- Proesmans, W., Smagghe, G., Meeus, I., Bonte, D., & Verheyen, K. (2019). The effect of mass-flowering orchards and semi-natural habitat on bumblebee colony performance. *Landscape Ecology*, 34(5), 1033–1044.
- Rader, R., Birkhofer, K., Schmucki, R., Smith, H. G., Stjernman, M., & Lindborg, R. (2014). Organic farming and heterogeneous landscapes positively affect different measures of plant diversity. *Journal of Applied Ecology*, 51(6), 1544–1553.
- Rattigan, K., & Hill, S. J. (1986). Relationship between temperature and flowering in almond. *Australian Journal of Experimental Agriculture*, 26(3), 399–404.
- Requier, F., Odoux, J.-F., Tamic, T., Moreau, N., Henry, M., Decourtye, A., & Bretagnolle, V. (2015). Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds. *Ecological Applications*, 25(4), 881–890.
- Salgado-Labouriau, M. L. (1973). *Contribuição à palinologia dos cerrados*. Academia Brasileira de Ciências Rio de Janeiro.
- Schmickl, T., & Crailsheim, K. (2004). Inner nest homeostasis in a changing environment with special emphasis on honey bee brood nursing and pollen supply. *Apidologie*, 35(3), 249–263.
- Schmidt, J. O. (1984). Feeding preferences of *Apis mellifera* L. (Hymenoptera: Apidae): Individual versus mixed pollen species. *Journal of the Kansas Entomological Society*, 323–327.
- Seeley, T. D., & Visscher, P. K. (1988). Assessing the benefits of cooperation in honeybee foraging: Search costs, forage quality, and competitive ability. *Behavioral Ecology and Sociobiology*, 22(4), 229–237.
- Southwick, E. E., & Southwick, L., Jr (1992). Estimating the economic value of honey bees (Hymenoptera: Apidae) as agricultural pollinators in the United States. *Journal of Economic Entomology*, 85(3), 621–633.
- Todd, F. E., & Bretherick, O. (1942). The composition of pollens. *Journal of Economic Entomology*, 35(3), 312–317.
- Williams, N. M., & Kremen, C. (2007). Resource distributions among habitats determine solitary bee offspring production in a mosaic landscape. *Ecological Applications*, 17(3), 910–921.
- Wirthensohn, M., Rahemi, M., & Fernández i Martí, A. (2011). Identification of self-incompatibility genotypes and dna fingerprinting of some australian almond cultivars. *Acta Horticulturae*, 912, 561–566. doi:10.17660/ActaHortic.2011.912.84.
- Woodcock, B. A., Edwards, M., Redhead, J., Meek, W. R., Nuttall, P., Falk, S., Nowakowski, M., & Pywell, R. F. (2013). Crop flower visitation by honeybees, bumblebees and solitary bees: Behavioural differences and diversity responses to landscape. *Agriculture, Ecosystems and Environment*, 171, 1–8. doi:10.1016/j.agee.2013.03.005 Scopus.
- Wratten, S. D., Gillespie, M., Decourtye, A., Mader, E., & Desneux, N. (2012). Pollinator habitat enhancement: Benefits to other ecosystem services. *Agriculture, Ecosystems & Environment*, 159, 112–122.