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RESOURCE AVAILABILITY INFLUENCES BEE INTERACTIONS WITH PARASITES, PATHOGENS, AND MICROBES IN AGRICULTURAL LANDSCAPES

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ABSTRACT

RESOURCE AVAILABILITY INFLUENCES BEE INTERACTIONS WITH PARASITES, PATHOGENS, AND MICROBES IN AGRICULTURAL LANDSACPES

Hamutahl Cohen

Bee populations are declining but bees are critically important for pollination. Through resource provisioning, landscape context impacts bees: bees with access to food and habitat are healthier. But landscape context also has epidemiological importance for bees. As a bee forages across landscapes for food, it acquires flower-associated microorganisms. These can be beneficial or pathogenic. Variation in human-managed landscapes may therefore influence bee health. This dissertation addresses whether and how resource availability and landscape composition in urban agricultural systems influence disease dynamics and microbiome composition in three species of domesticated bees with wild counterparts: orchard bees, bumble bees, and honey bees.

The research was conducted in 18-25 urban gardens along the central coast of California. Differences at these gardens in terms of local (such as crop diversity) and landscape features (such natural cover) allowed me to ask how landscape processes such as urbanization impact bee health. In the 1st and 2nd chapter, I examine how garden management influences parasite and pathogen prevalence. In the 3rd chapter, I compare microbiome composition between orchard bees. In the 4th chapter, I use

qualitative methods to describe the social factors shaping sustainable beekeeping practices.

I found that floral resources in urban gardens are positively associated with the prevalence of *Apicystis* and *A. borealis* in honey bees and with the prevalence of Deformed Wing Virus and Acute Bee Paralysis Virus in *Bombus vosnesenskii*. While these findings suggest that floral resources in urban contexts may amplify disease risk, I also found that nesting site availability (bare soil) negatively predicts the prevalence of some parasites and pathogens in bumble bees. I suggest more research on the tradeoffs between resource provisioning and parasite and pathogen transmission. Furthermore, while floral resources were associated with disease transmission, they were also associated with the abundance of bacterial groups beneficial to bee health. In *Osmia lignaria,* floral abundance was correlated with *Lactobacillus*, which was associated with reduced *Crithidia* prevalence. These studies highlight complex interactions between environmental context, bee diversity, and bee-associated microbes. I contextualize these findings in a qualitative beekeeping study, suggesting that the unique features of the beekeeping industry can influence the outcomes of resource provisioning on bee health.

DEDICATION

To my parents

"C'mo avoti natu bishvili, kach ani listol l'yiladi"- Taanit 23a

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CHAPTER 1: Vegetation management and host density influence bee-parasite interactions in urban gardens

Abstract

Apocephalus borealis phorid flies, a parasitoid of bumble bees and yellow jacket wasps in North America, was recently reported as a novel parasitoid of the honey bee *Apis mellifera*. Little is known about the ecology of this interaction, including phorid fecundity on bee hosts, whether phorid-bee parasitism is density dependent, and which local habitat and landscape features may correlate with changes in parasitism rates for either bumble or honey bees. We examined the impact of local and landscape drivers and host abundance on phorid parasitism of *A. mellifera* and the bumble bee *Bombus vosnesenskii*. We worked in 19 urban gardens along the North-Central Coast of California, where phorid parasitism of honey bees was first reported in 2012. We collected and incubated bees for phorid emergence, and surveyed local vegetation, ground cover, and floral characteristics as well as land cover types surrounding gardens. We found that phorid parasitism was higher on bumble bees than on honey bees, and phorids produced nearly twice as many pupae on individual bumble bee hosts than on honey bee hosts. Parasitism of both bumble and honey bees increased with abundance of honey bees in a site. Differences in landscape surroundings did not correlate with parasitism, but local factors related to bee resource provisioning (e.g. tree and shrub abundance) positively correlated with increased parasitism. This research thus helps to document and describe conditions

that may have facilitated phorid fly host shift to honey bees and further elucidate how resource provisioning in urban gardens influences bee-parasite interactions.

Introduction

Honey and bumble bee populations are declining in several regions, with negative implications for ecosystem services (Potts et al. 2010). In 2014-2015, commercial beekeepers lost 42.1% of managed honey bee (*Apis mellifera)* hives, an increase from the losses of 2013-2014 and the second highest annual loss to-date (USDA 2015). Bee decline is troublesome because bees are critical pollinators in many agricultural ecosystems, with 35% of global crops depending on pollination (Klein et al. 2007). With the mounting concern around honey bee losses, many have looked to wild and domesticated bee species (such as bumble bees, *Bombus* spp.) for insurance against pollination losses (Buchmann and Nabham 2012). Domesticated bumble bees are widely used in commercial agriculture and for some crops are more efficient pollinators than honeybees (e.g. Stubbs and Drummond 2001; Li et al. 2006). However, bumble bee populations are also declining. In Europe, many bumble bee species have experienced range contractions and localized extinctions (Goulson et al. 2008; Kosior et al. 2007). In North America, formerly abundant and widespread bumble bee species have declined since the late 1990s, with some species presumed extinct (Williams and Osborne 2009; Gritxti et al. 2009). There is also evidence of bumble bee population decline and species richness loss in South America (Schmid-

Hempel et al. 2014), China (Xie et al. 2008, Williams et al. 2009), and Japan (Matsumura et al. 2004; Inoue et al. 2008).

Bumble bee and honey bee decline can be attributed to an overlapping set of stressors including parasites, pathogens, and land-use change (Grixti et al. 2009; Potts et al. 2010, Goulson et al. 2015). Bees host a broad range of parasites and parasitoids that can negatively affect their populations. Several parasites (including *Nosema* spp*., Apicystis bombi*, and *Crithidia* spp.) are implicated in the decline of honey bees and commercial *Bombus terrestris* (Cameron et al. 2011; Graystock et al. 2013). While honey bee parasites have received attention for their role in colony loss (e.g. Cox-Foster et al. 2007, Nazzi et al. 2012), our knowledge of parasites and parasitoids for wild bee species is more limited. Nevertheless, information about honey bees parasites and parasitoids may be relevant to understanding wild bumble bee declines because many honey bee parasites such as *Nosema ceranae* (Graystock et al. 2013) infect both honey bees and bumble bees (Fürst et al. 2014, McMahon et al. 2015). Furthermore infection by parasites and parasitoids has been shown to impact bumble bee foraging patterns, behavior, and physiology (König and Hempel 1995, Müller 1994, Schmid-Hempel and Schmid-Hempel 1998)

In addition, land-use change at local and landscape scales may negatively impact bee populations. Several qualitative syntheses suggest that agricultural intensification and habitat fragmentation negatively affect bee abundance and diversity (e.g. Ricketts et al. 2008, Winfree et al. 2009). This is because changes in urban cover, natural woodlands, or open space within a landscape, as well as changes

in vegetation structure within a site, can alter the habitat and food resources available to bees with population-level impacts (Kremen et al. 2007). Interactions between land-use change and parasites and pathogens may be important for bee decline (Paxton et al. 2016, Botías et al. 2017). When landscape processes such as urbanization or conversion of natural habitat to intensive agriculture alter the availability of food and habitat resources of a host, there can be one of two outcomes for parasitism: dilution or amplification (Becker et al. 2015). Dilution may occur when land-use change increases the quality and quantity of food and habitat resources for a host, resulting in higher immunity or defense against parasitism. Amplification may occur if increase in resources in the landscape results in host aggregation, increasing parasite transmission between individuals (Becker et al. 2015). Resource mediated dilution has been observed in lace monitor reptiles for which increased access to urban waste improves nutrition and lowers parasite intensity (Jessop et al. 2012), in long-tailed macaques with higher access to nutrition and lower *Giardia* infection (Lane et al. 2011), and in other non-arthropod systems (Becker et al. 2015). Amplification due to resource provisioning has been observed for infection of Elk by *Brucella abortis* (Cross et al. 2007) and infection of White-tailed deer by *Mycobacterium bovis* (Miller et al. 2003). Yet, very little is known about how resource or habitat changes alter bee-parasite interactions.

Urban gardens provide a unique environment to study how land use change and parasitism influence bees. Urban spaces are characterized by increases in impervious cover, structural simplification of vegetation and a heterogeneous mosaic

of land-use (Marzluff and Ewing 2001; Thompson et al. 2003). However, local and landscape features of urban gardens provide habitat and food resources for biodiversity, supporting pollinator populations and other beneficial insects (Goddard et al. 2010). Across multiple studies, floral abundance and richness in urban gardens promotes higher bee diversity (Pardee and Philpott 2014; Quistberg et al. 2016; Tommasi et al. 2004; Wojcik et al. 2008). In a review of urban bee ecology, Hernandez, Frankie and Thorp (Hernandez et al. 2009) found a negative correlation between bee species and urban development, although the effect of landscape variation can differ across scales (Pardee and Philpott 2014; Quistberg et al. 2016). These local and landscape characteristics of urban gardens may also impact insect-parasite and insect-parasitoid interactions. Urbanization-mediated resource shifts may result in changes to parasite and host geographic ranges and population densities, potentially leading to the emergence of key parasites and parasitoids, altered behavior for hosts, parasites, and parasitoids, and potentially higher infection rates (Bradley and Altizer 2007; Keesing et al. 20010; Becker et al. 2015). If urbanization results in biodiversity losses, shifts in species composition may also influence parasitism.

Here, we investigate parasitism of bees by *Apocephalus borealis* (Diptera: Phoridae), a phorid fly native to North America (Brown 1993). *A. borealis* phorids can reduce bee worker lifespan by up to 70% in the native host, the bumble bee (*Bombus* spp.) (Otterstatter et al. 2002). It is also a native parasitoid of the yellow jacket wasp (*Vespula spp*.) (Ennik 1973). Phorids in the genus *Apocephalus* are called "decapitating flies" and commonly have host associations with ant species (Brown

1997). Recently, researchers documented *A. borealis* phorids parasitizing honey bees (*Apis melifera*), a novel host-parasitoid interaction (Core et al. 2012). Phorid-infected honey bees are described as "zombie bees" because they may show hive abandonment behavior at night. Multiple phorid larvae develop from each bee host, feeding on thoracic flight muscle (Ennik 1973). Larve then emerge to pupate, although this has only been observed in-vitro (Core et al. 2012). The dispersal range of *A. borealis* is unknown, although phorid in the genus *Pseudacteon* have been shown to disperse 650m away from hosts (Morrison et al. 1999). Research on phorid parasitism of honey bees and bumble bees is limited to a few studies examining prevalence and mortality rates in bees (e.g. Core et al. 2012; Otterstatter et al. 2002) and an on-going citizen science project (zombeewatch.org) examining the distribution of phorid parasites in bees across the US.

One of the unknowns about phorid-bee interactions is whether honey bees are a native host previously undiscovered for *A. borealis* or whether *A. borealis* phorid flies have recently extended their host range to include honey bees. Understanding host choice is important because changes in host size for other arthropod parasites influences behavioral, morphological, and life history traits of the parasitoid (Messina 2004). Another unknown about this system is whether parasitism is spatially density dependent. While it is commonly assumed that parasitism rate and host density are positively correlated, this it not always the case because of limits to parasitoid ability to search for and handle hosts (Walde and Murdoch 1988). Understanding the

ecology of this novel parasitoid – host system is important for diagnosing what factors mediate parasitism rates in for vulnerable bee populations.

In this study, we examined bee-phorid interactions in an array of urban gardens that differ in bee abundance, local habitat conditions, and landscape surroundings. We examined if bee abundance influences parasitism by the phorid fly. We also asked if parasitized honey bees are host to a greater number of phorid pupae than bumble bees. To determine if resource provisioning influences parasitism rates, we examined the influences of local vegetation change and landscape-level land-cover change on parasitism of *Apis mellifera* and *Bombus vosnesenskeii* by phorid parasitoids. For some phorid-host interactions (e.g. with ant hosts), effects on hosts may differ with land-use change (e.g. agricultural management) (Pardee and Philpott 2011, De la Mora et al. 2015) but little is known about how land-use change may alter bee-phorid interactions, specifically. We asked, 1) Does the number of pupae emerging from *Apis mellifera* and *Bombus vosnesenskeii* individuals differ? 2) How do parasitism rates of *Apis mellifera* and *Bombus vosnesenskeii* differ depending on the abundance of honey bees and bumble bees within garden sites? 3) Which local and landscape level factors influence parasitism rates in honey bees and bumble bees? We expected more pupae in *Bombus vosnesenskeii* hosts because they are large-bodied bees, and a native host of the phorid. We expected more phorid infection in sites with more honey bees and bumble bees because phorids and other parasitoids often exhibit density dependence with hosts (e.g. Philpott et al. 2009). Finally, we expected lower phorid parasitism in sites with higher local vegetative diversity and

lower urban landscape cover due to potential nutrition benefits to bees and subsequent dilution effects.

Methods

Characterization of the study sites

Between June and October 2014 we examined local and landscape characteristics of 19 urban gardens, ranging in size from 444 m^2 to 15,525 m^2, each separated by 2 km, across three counties (Monterey, Santa Clara, and Santa Cruz) in the California central coast (Fig. 1.1). We measured local habitat characteristics (e.g. vegetation and ground cover) five times within a 20 x 20 m plot placed at the center of each garden. We measured canopy cover with a convex spherical densitometer at the center of the plot, and 10 m to the N, S, E, and W. We counted and identified all trees and shrubs and noted the number of individuals in flower. In each plot, we randomly selected four 1 x 1 m plots within which we identified all herbaceous plants (except grasses) to morphospecies, measured height of the tallest non-woody vegetation, counted flowers, and assessed percent ground cover from bare soil, grass, herbaceous plants, leaf litter, rocks, and mulch. In a 100 × 100 m plot surrounding each garden, we counted all trees and quantified percent area with concrete and buildings, mulch, lawn, woody vegetation, weedy or non-woody vegetation, and bare ground. For analysis, values were averaged across the five sample dates. We also estimated the total garden size. Overall, we measured 21 local habitat variables (Table 1).

At the landscape scale, we classified land cover types within 2 km buffers surrounding each garden with data from the 2011 National Land Cover Database (NLCD, 30 m resolution) (Homer et al. 2011). We selected 2 km buffers as most bees forage within 2 km from a nesting site (Kremen et al. 2004). While honey bees have a large maximum foraging distance, foraging distance varies as a function of landscape context (Steffan-Dewenter and Kuhn 2003) and mean foraging distances for honey bees have been reported at approximately 1km (Waddington et al. 1994, Beekman and Ratnieks 2000, Schneider and Hall 1997) and 2.3km (Visscher and Seeley 1982). *Bombus vosnesenskii* has a predicted maximum foraging distance of 2.1km (Greenleaf and Kremen 2006), and some fly parasitoids in the family Phoridae are known to disperse under 1km (Morrison et al. 1995). We created four land-use categories: 1) natural habitat (deciduous [NLCD number 41], evergreen [42], and mixed forests [43], dwarf scrub [51], shrub/scrub [52], and grassland/herbaceous [71]), 2) open (lawn grass, parks, and golf courses [21]), 3) urban (low [22], medium [23], and high intensity developed land [24]), and 4) agriculture (pasture/hay [81] and cultivated crop [82]). Other land cover types covered <5% of the total area and were not included. We assessed land cover with spatial statistics tools in ArcGIS v. 10.1.

Bee collection and parasitism assessment

We collected bees (*B. vosnesenskeii* and *A. mellifera*) at each site and incubated bees in the lab to assess phorid emergence. We netted bees along walking transects in 20 x 20 m plots (and within 20 m of plots) for 30 min every 3 weeks

between mid-June and early-October 2014, for a total of 6 sampling periods. Bees were captured alive, placed in individual rearing containers, and observed for pupae to determine parasitism. Bees were held at in terrariums under heat lamps between 72-74°F and monitored daily for 10 d for phorid emergence. We recorded number of pupae that emerged from each infected bee.

Data analysis

Because many explanatory variables measured may be correlated, we divided most variables into four biologically relevant groups (ground cover in 20 x 20 m plots, ground cover in 100 x 100 m plots, tree and shrub characteristics, and landscape characteristics) and ran Pearson's correlations to identify correlated ($P<0.01$) variables within groups. We selected variables that were correlated with the largest number of other variables in that group for subsequent analysis (Table 1.1). Four variables (no. of flowers, height of tallest vegetation, herbaceous plant richness, and garden size) were not put into any group, and were also included (a). In all, we included 10 local vegetation variables and two landscape variables for subsequent analyses.

In order to examine differences in the number of pupae emerging from honey bees and bumble bees, we compared the mean number of pupae per parasitized individual with t-tests assuming unequal variance in Excel. In order to examine density dependence of phorid infection, we fit linear models using the lm function in R. To examine the relationship between sampling period and phorid infection, we fit

a generalized linear mixed model of infection rates with site as a random effect and sampling period as a fixed effect using the glmer function using the lme4 package in R. Because the data were not normally distributed, we used the 'cbind' function, a binomial error distribution, and logit link (Warton and Hui 2011). For the local and landscape features, we did not include sampling period in our model because changes over the summer in vegetation at the field sites did not change drastically.

We used generalized linear models (GLMs) with the glm function in R (Team RC 2014) to examine relationships between bee abundance (*A. mellifera* and *B. vosnesenskii* abundance) selected site variables (local variables, landscape variables) and the percent of *A. mellifera* and *B. vosnesenskii* infected by *A. borealis* phorids at each site. When the percent of individual individuals of each bee was averaged across time periods, the data were normally distributed. We tested all combinations of the 14 selected variables with the 'glmulti' package (Calcagno and de Mazancourt 2010) and selected the top model based on the AICc values. For models where the AICc for top models was within 2 points of the next best model, we ran model averages with the MuMIn package (Barton 2012). For both *A. mellifera* and *B. vosnesenskii*, the best models shared the same significant predictors as model averages, and thus we report output from best models only. Dependent and predictor variables were normally distributed, so we used a Gaussian error structure for GLMs, and report AICc values, and p-values. To determine the goodness-of-fit of the best models, we calculated a pseudo-R^2 value as [(null deviance - residual deviance)/ null deviance] (Dobson and Barnett 2008). All residuals from best models conformed to the conditions of

normality as checked with QQ-Plots and Shapiro-Wilk tests.

Results

Phorids were more likely to parasitize bumble bees and bumble bees supported higher pupae loads. Of the 1819 *A. mellifera* individuals we collected, 30 were parasitized. Of the 290 *B. vosnesenskeii* individuals we collected, 17 were parasitized. Across all garden sites, an average of 0.77% ± .33 (SE) *A. mellifera* and 4.53% ± 1.99 *B. vosnesenskeii* individuals were parasitized. Of all urban garden sites sampled, 37% contained parasitized honey bees and 21% contained parasitized bumble bees. Infected bees were found in coastal sites in Monterey and Santa Cruz county, but not present in inland sites in Santa Clara County (Fig 1.1; Table 1.2). When site was taken into account as a random factor, however, sampling period had no impact on infection rates for honey bees ($z=1.46$, $p=0.14$) and bumble bees ($z=-0.271$, $p=0.79$). There were nearly twice as many pupae per bumble bee (7.26 ± 0.87 SE) than per honey bee (4.23 ± 0.46 SE) in the parasitized individuals collected ($t=3.076$, $p=0.0046$).

Phorid parasitism in both *A. mellifera* ($p=0.00081$) and *B. vosnesenskei* ($p=0.012$) increased with the abundance of *A. mellifera* in a site. In contrast, phorid parasitism did not differ with increasing *B. vosnesenskei abundance* for either *A. mellifera or B. vosnessenkii* ($p<0.05$).

Parasitism of both species of bees responded to local, but not landscape variables. The model that best predicted *A. mellifera* parasitism included garden size,

the number of trees and shrubs, the percent lawn within a 100 x 100 m plot, the average percent of bare soil in 20 x 20 m plots, and the average abundance of *B. vosnesenskii* collected per sampling period (AICc = 35.86, df = 18, pseudo-R^2 = 0.945). Parasitism of *A. mellifera* was higher in gardens with more trees and shrubs (P < 0.0001) in larger gardens (P < 0.0001), with more lawn (P = 0.005), in gardens with less bare soil (P=0.004), and in gardens with lower *B. vosnesenskii* abundance (P = 0.001)(Fig. 1.2). The model that best predicted *B. vosnesenskii* parasitism included the number of trees and shrubs and percent of bare soil in 20 x 20 m plots (AICc = 128.265, df = 18, pseudo-R^2 = 0.604). *B. vosnesenskii* parasitism was higher in gardens with more trees and shrubs (P=0.006) and in gardens with less bare soil (P=0.011)(Fig. 1.3).

Discussion

Core et al. hypothesize that parasitism by *A. borealis* phorids in honey bees reflects a recent host shift from their native host, the bumble bee (2012). Possible reasons for a host shift include a benefit to phorid fitness due to increased parasitism success with a new host or increased abundance of an alternative host. To examine if phorids experience increased parasitism success with host choice, we compared the mean number of pupae emerging from bumble bees and honey bees and found that the mean number of pupae emerging from each infected bumble bee (7.26 ± 0.87 SE) was significantly higher (by nearly twice) than the number of pupae emerging from each honey bee (4.23 ± 0.46 SE). Our finding that bumble bees are host to more fly

progeny is consistent with previously published studies: Otterstatter et al. (2002) found an average of 6.57 fly larvae per female bumble bee and Core et al. (2012) found an average of 4.8 fly larvae in honey bee females. Honey bee bodies may be hospitable to fewer phorid pupae because they are smaller than bumble bee bodies, suggesting a loss to phorid fitness and implying that there may be other reasons for a host shift to honey bees. In this study, honey bees out-numbered bumblebees in our field collection by approximately 6 to 1. Even though phorids produce less offspring in honey bee hosts, a phorid facing a multitude of available honey bee hosts may counter a reduction to fitness by parasitizing more honey bees in her lifetime than bumblebees. Infecting two honey bees would, on average, produce more phorid offspring than a single bee. Future experiments determining how many honey bee hosts a single phorid female parasitizes in her lifetime may help explain why phorids infect honey bees. Another important question is whether honey bees and bumble bees are only parasitized by one phorid or by multiple phorids, as this can impact the fitness tradeoff between host availability and pupal load per bee.

With declines of bumble bee populations reported in North America (Cameron et al. 2011), a possible explanation for a host shift to honey bees may be a loss of access to bumble bee hosts. Indeed, we were able to collect more honey bees than bumble bees during field collections, and more likely to find honey bees infected with phorids than bumble bees infected with phorids. As the six sampling periods progressed, we caught fewer bumble bees. When we stopped finding bumble bees we saw a slight increase in the percent of honey bees parasitized by phorids (Fig. 1.4).

Otterstater et al. (2002) and Core et al. (2012) previously found that phorid infection of bees increased between May and August. However, we did not find a significant impact of season on infection, possibly because we found so few bumble bees at the end of our sampling season or because our collection period started 30 days later than previous studies. In addition to sampling period, another driver of phorid infection might be temperature and climate. We did not find infected bees from the very high-temperature inland sites in Santa Clara. When infected bees were present, they were found in sites along the cooler Monterey and Santa Cruz coastal region (Fig.1.1). And previously studied *A. borealis* phorids were also found in coastal regions or near bodies of water (Core et al. 2012, Otterstater et al. 2002).

Phorid flies in urban settings respond to population-level changes in host abundance: we found that increased abundance of honey bees, but not bumble bees, correlated with higher parasitism of both honey bees and bumble bees. One possible explanation may be that honey bees may be easier to locate or parasitize than bumble bees, but little is known about host location in bee-phorid interactions, as Phoridae is one of the least studied groups of Dipterans (Brown 2004). Previous studies have also noted that density dependence may be temporally or spatially scale dependent (Philpott et al. 2009). In this study, we found that phorid parasitism is density dependent at the garden scale - but interestingly only for abundance of the domesticated, non-native host (*A. mellifera*). The abundance of the non-native honey bee may contribute to bumble bee infection and decline. Even if honey bees are infected with phorids, their numbers are often re-established by beekeepers, keeping

the honey bee population high even in the face of increased levels of parasitism. Indeed, beekeeping is highly popular and practiced widely in our region of study, both by commercial and backyard beekeepers. Because of this, bumble bees might continue to encounter large numbers of phorid parasitoids even as their availability as a host declines. Overall, more research is required to determine when and why *A. borealis* phorids began to shift bee hosts, under which conditions parasitism of honey bees and bumble bees is more likely, and what the impacts might be to native bumble bee populations. This is important because parasitoids can mediate the composition and dynamics of communities (Feender 2002).

We found that differences in parasitism of both *A. mellifera* and *Bombus vosnesenskii* were driven by local features of urban gardens, not landscape factors. The local variables that correlate with parasitism, tree and shrub abundance, bare soil, lawn, and garden size, may directly or indirectly benefits phorids in some way. These factors, however, are also indicators of resource availability for bees. Trees and shrubs include flowering species that provide pollen and nectar for food, larger gardens may provide more habitat than smaller gardens, and bare ground has been associated with increases in ground-nesting bee populations (Potts et al. 2005; Quistberg et al. 2016). Even the amount of lawns in a given area has been shown to be an important food source for bees due to the presence of flowering weeds (Larson et al. 2014). Garden size was a positive indicator of parasitism for only of *A. mellifera*. Garden size may be an important indicator of resource availability for *A. mellifera* because honey bees have been said to forage across long distances, up to 20

km, but their foraging is influenced by resource availability (Steffan-Dewenter and Kuhn 2003). For a honey bee foraging across long distances, larger gardens may host more resources and therefore attract more visitation than smaller gardens. Comparatively, *B. vosnesenskii* have recorded foraging distances of 0.8 - 2.8 km (Jha and Kremen 2013), suggesting that those bees collected for study are somewhat more likely to have nested locally and that garden size may not be as critical an indicator as resource availability. If local habitat features are responsible for parasitism increases through indirect impacts to bees, then our results support the idea that resource provisioning results in amplification of parasitism. As larger resource-rich urban gardens with more food and habitat attract more bees, this may result in increased contact rates between hosts and phorid parasites. Another interesting finding was that, in modeling the combined influence of vegetation and bee diversity on parasitism, the average abundance of *B. vosnesenskii* individuals in a garden was a negative predictor of the number of infected *A. mellifera* individuals. This may have to do with competitive interactions between bumble bees and honey bees for resources, phorid host preferences, or point to complicated relationships between vegetative resources, bee community composition, and parasitism.

We initially expected that habitats with high floral diversity would provide resources for bees, conferring immunological or physiological defense against phorid parasitism. In lab experiments, bees fed poly-floral pollen diets showed higher expression of immune-related genes and lower mortality when challenged by *Nosema* parasites than bees fed mono-floral diets (Di Pasquale et al. 2013). Further, chemical

constituents of pollen and honey up-regulate select detoxification and antimicrobial peptide genes related to immunity (Mao et al. 2013). However, we did not find any influence (either positive or negative) of floral abundance on parasitism rates. One explanation may be that honey bees and bumble bees are simply not able to overcome phorid infection through immunological defense, regardless of resource availability. Additionally, because phorid adults also consume pollen and nectar diets (Nicolson 2007) and because increased food provisioning has been shown to promote phorid longevity (Wäckers 2001), any immunity or nutritional benefits conferred to the bee may be obscured by increased resource availability for phorids. Whether or not resource availability dilutes parasitism may thus be a function of the availability of preferred floral resources for phorid parasitoids, a factor that is still virtually unknown for many phorids (Wäckers and Fadamiro 2005).

Because phorid parasitism is density dependent in this system, possible increases to bee richness and abundance due to changes to resources may amplify phorid detection of bee hosts or increase contact rates between infected bees. Ultimately, deciphering the relationship between local and landscape features and epidemiology of parasitic infections is complex: beyond resource provisioning, landscape can exert direct influences on host or parasite growth, through indirect impacts to host and parasite physiology and behavior, through alterations to host community structure, and more (Kilpatrick and Altizer 2010). Further studies, including both laboratory and field observation research, are needed to tease apart the complex interactions between resource provisioning and parasitism. The first step

may be to learn more about *A. borealis* ecology. By understanding phorid habitat, food, and microclimate preferences, how and where they locate and parasitize bees, we may elucidate how phorid-bee interactions are impacted by resource availability, bee diversity, and landscape context.

Authors' contributions

Hamutahl Cohen acquired funding for the research, led study design, fieldwork, lab work, and coordinated manuscript writing and publication. Co-author Robyn Quistberg contributed to experimental field design, laboratory processing of samples, and contributed to the manuscript. Co-author Stacy M. Philpott contributed to field research design, fieldwork logistics, data analysis, and manuscript writing. (See author permissions in supplemental files).

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Table 1.1 Results of Pearson's correlations showing groups of explanatory variables, variables selected for GLM analysis, and variables correlated ($p<0.01$) with selected variables (n=19).

Group	**Selected Variables**	**Correlated variable ($p<0.01$)**	**Correlation coefficient**	**Direction of Correlation**
Ground cover (20x20 m plots)	Grass	NA	NA	NA
	Litter	NA	NA	NA
Ground cover (100x100m)	Concrete and buildings	Weedy, non-woody vegetation	0.684	-
		Mulch	0.561	-
	Lawn	NA	NA	NA
	Bare	NA	NA	NA
Tree and shrub characteristics	No. trees and shrubs	Canopy cover	0.919	+
		No. tree and shrub species	0.793	+
		No. trees and shrubs in flower	0.83	+
		No. trees and shrubs	0.597	+
		Woody cover	0.632	+
Landscape characteristics	Natural 2 km	Urban 2 km	0.912	-
		Open 2 km	0.777	-
	Agriculture 2 km	NA	NA	NA
Non-grouped characteristics	No. of flowers	NA	NA	NA
	Height of tallest vegetation	NA	NA	NA
	Herbaceous plant richness	NA	NA	NA
	Garden Size	NA	NA	NA

Table 1.2 The mean and standard deviation of infection rate for each species by garden site. Garden sites are numbered 1-19 to maintain confidentiality.

		Honey bee		Bumble bee	
Garden	County	Mean (%)	SD	Mean (%)	SD
Garden 1	Santa Cruz	1.866	3.188	0.000	0.000
Garden 2	Santa Cruz	1.111	2.485	0.000	0.000
Garden 3	Santa Cruz	4.505	3.184	26.111	37.536
Garden 4	Santa Cruz	3.687	5.200	7.176	13.712
Garden 5	Santa Cruz	1.754	3.923	19.444	36.536
Garden 6	Santa Cruz	4.627	4.402	19.697	36.521
Garden 7	Santa Cruz	0.000	0.000	0.000	0.000
Garden 8	Santa Cruz	0.000	0.000	0.000	0.000
Garden 9	Monterey	2.924	3.269	2.778	6.211
Garden 10	Monterey	0.000	0.000	0.000	0.000
Garden 11	Monterey	0.000	0.000	0.000	0.000
Garden 12	Monterey	0.000	0.000	0.000	0.000
Garden 13	Monterey	0.000	0.000	0.000	0.000
Garden 14	Monterey	0.000	0.000	0.000	0.000
Garden 15	Santa Clara	0.000	0.000	0.000	0.000
Garden 16	Santa Clara	0.000	0.000	0.000	0.000
Garden 17	Santa Clara	0.000	0.000	0.000	0.000
Garden 18	Santa Clara	0.000	0.000	0.000	0.000
Garden 19	Santa Clara	0.000	0.000	0.000	0.000

Figure 1.1 Map of urban garden field sites in along the central coast of California. Each dot represents an urban garden field site in which there was no parasitism, parasitism of *Apis mellifer*a bees, or parasitism of *Bombus vosnesenskii*.

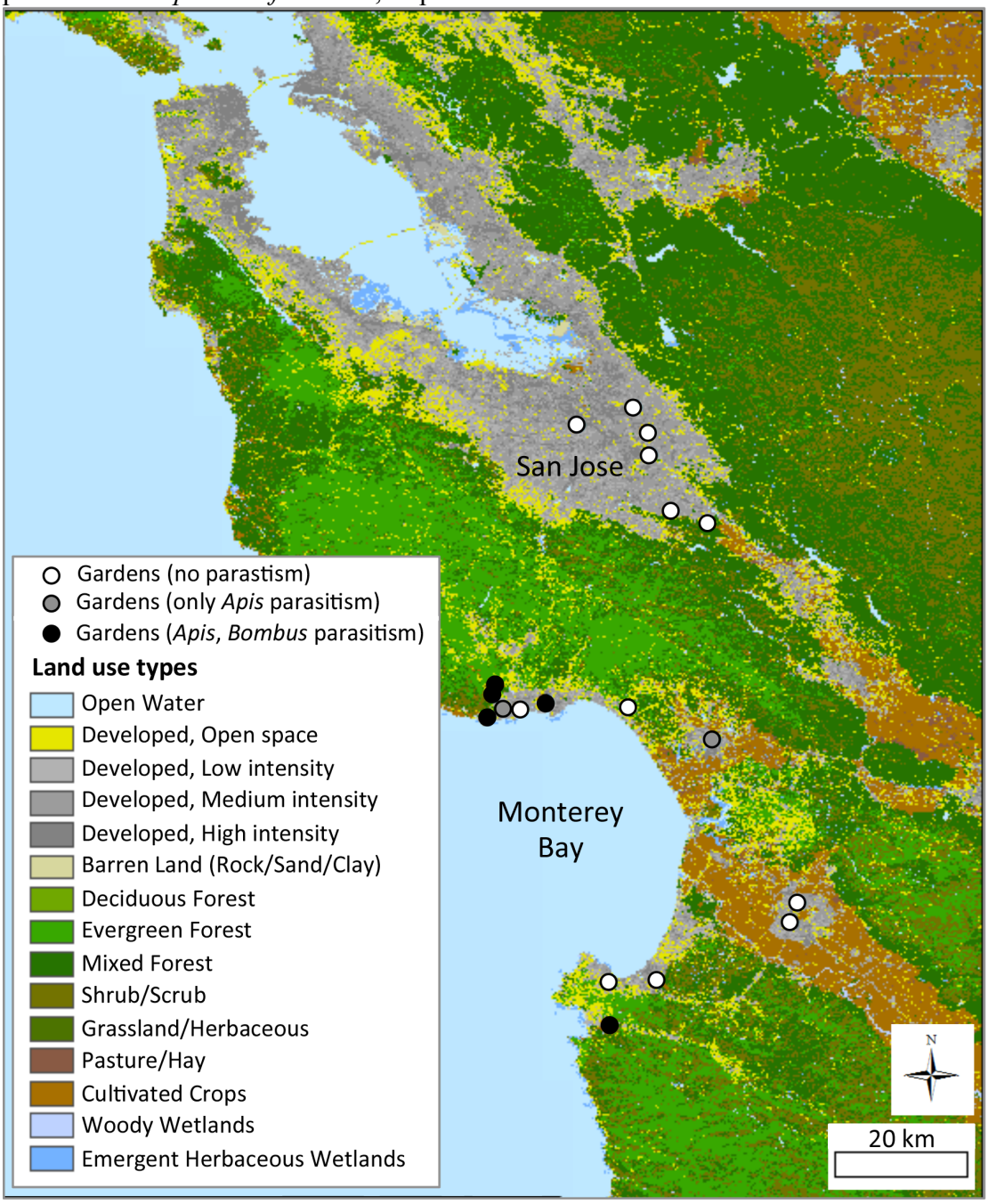


Figure 1.2 Relationships between significant factors in generalized linear models and parasitism of honey bees by *A. borealis* (a-e). Each dot represents an urban garden field sites. Lines show the best fit, and grey area reflects confidence bands of the generalized linear models.

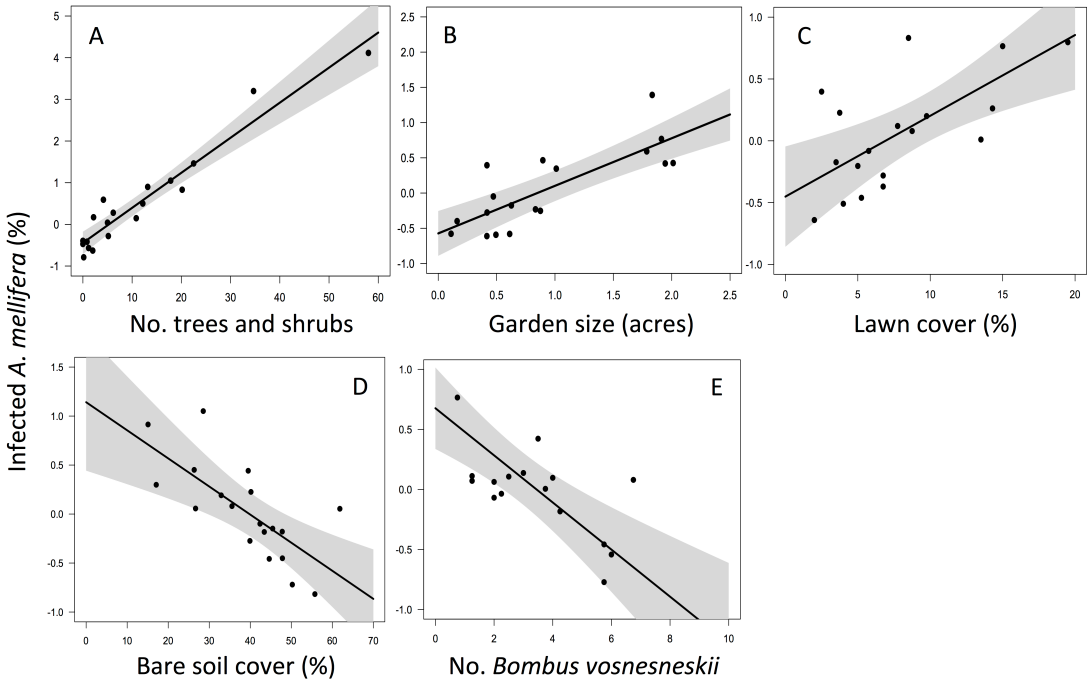


Figure 1.3 Relationships between significant factors in generalized linear models and parasitism of bumble bees bees by *A. borealis* (a,b). Each dot represents an urban garden field sites. Lines show the best fit, and grey area reflects confidence bands of the generalized linear models.

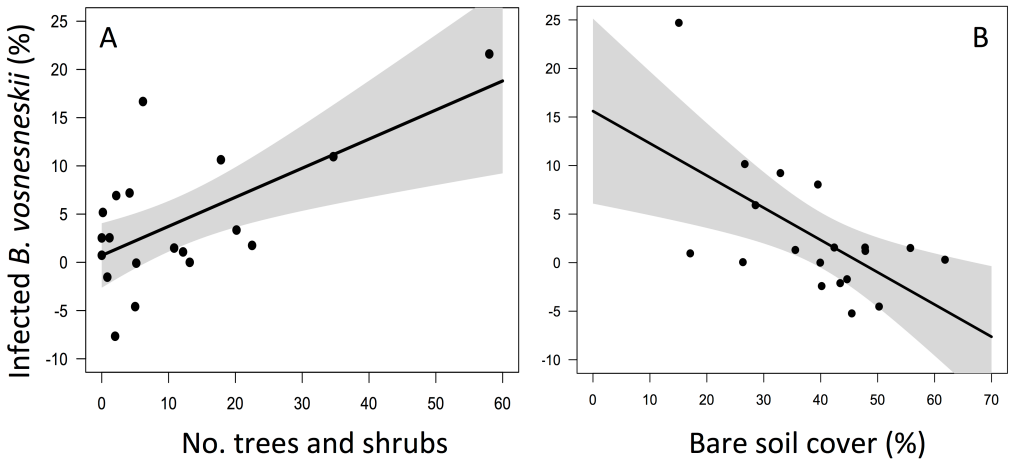
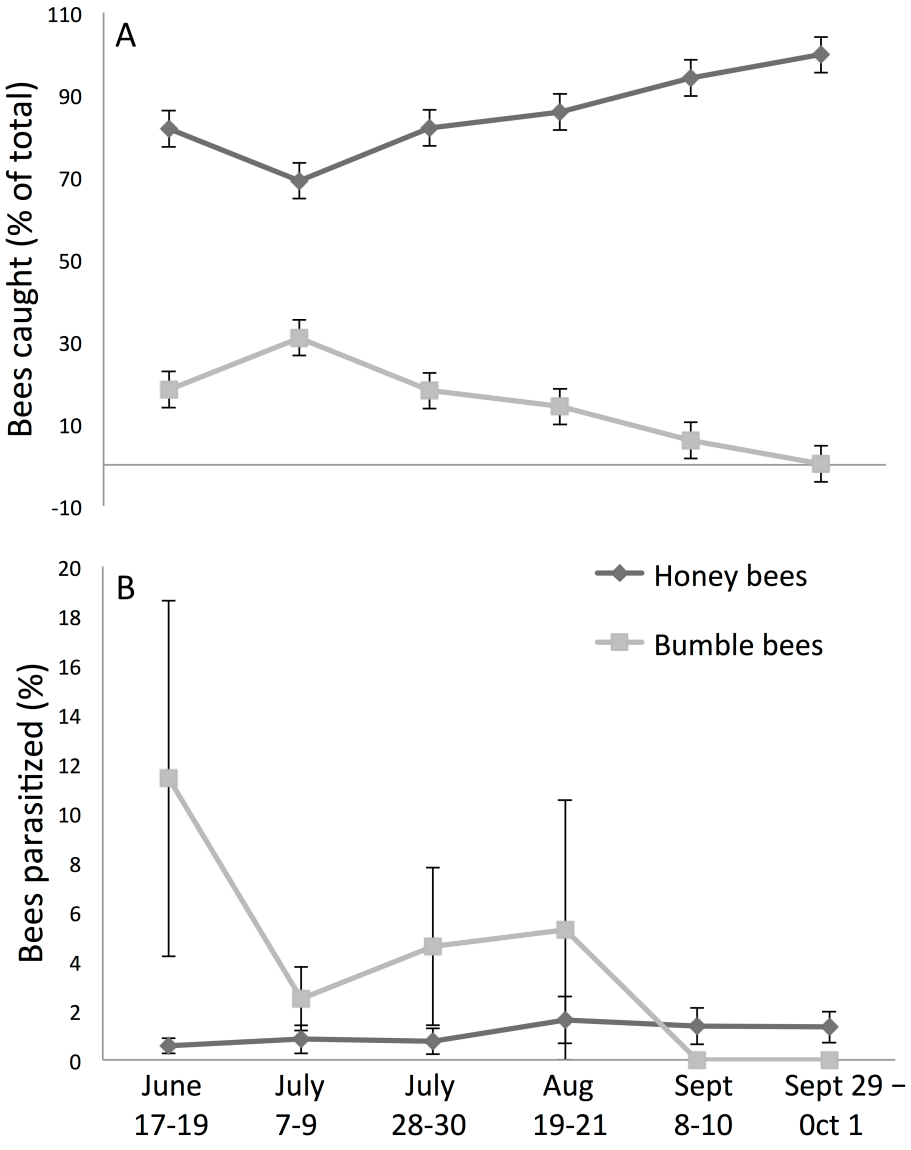


Figure 1.4 Seasonal changes across 2014 in a) the average percent of total captures from urban gardens that were honey bees or bumble bees, and b) the average percent of honey bees and bumble bees captured at each site that were parasitized by phorid flies



CHAPTER 2: Floral resource provisioning in urban gardens amplifies parasite and pathogen risk for bees.

Abstract

In human-dominated urban landscapes, it is widely believed that declining bee populations benefit from floral resource provisioning. But floral resources influence disease dynamics because many parasites and pathogens are associated with pollen and nectar. In 18 urban gardens, we assessed the prevalence of three parasites and a suite of RNA viruses in honey bees and bumble bees to examine how urban garden management and bee community composition influence parasite and pathogen prevalence. We found that the abundance of floral resources in urban gardens was positively associated with the prevalence of *Apicystis* spp. and the phorid fly *Apocephalus borealis* in honey bees and positively associated with the prevalence of Deformed Wing Virus and Acute Bee Paralysis Virus in *Bombus vosnesenskii*. While these findings suggest that floral resources in urban contexts may amplify disease risk, we also found garden features associated with nesting site availability (bare soil cover) negatively predicts the prevalence of some parasites and pathogens in bumble bees. While floral resources provide bees with pollen and nectar food, our findings suggest more research is needed on the tradeoffs between resource provisioning and parasite and pathogen transmission.

Introduction

Urban gardens provide refuges for threatened wild bee populations (e.g. Colla et al. 2009, Hernandez et al. 2009, Goddard et al. 2010, Quistberg et al. 2016), but not all urban gardens are created equal. The conservation potential of an urban garden depends both on garden characteristics and landscape surroundings because bees require floral and nesting resources across temporal and spatial scales (Kremen et al. 2007). Garden features such as ground cover, floral and nesting resource availability, as well as landscape heterogeneity influence bee richness, abundance, and community composition (Matteson & Langellotto 2010, Bates et al. 2011, Pardee & Philpott 2014, Baldock et al. 2015, Potter & LeBuhn 2015, Quistberg et al. 2016, Plascencia & Philpott 2017). To advance pollinator conservation, researchers and gardeners have applied these findings, adding flowering plants or reserving undisturbed soil to provide floral and nesting resources for bees. There has been limited research on the outcomes of resource provisioning in gardens for bee diversity, but early studies indicate mixed success. In gardens that participate in public initiatives to provision for pollinators, enhancement of local floral diversity may outweigh the influence of existing landscape effects (Shwartz et al. 2013). Floral abundance may also increase pollinator activity in urban gardens (Wojcik et al. 2008, Fukase & Simon 2016, Simao et al. 2018), but may fail to increase bee richness (Matteson & Langellotto 2011, Plascencia & Philpott 2017). This study addresses these heterogeneous outcomes to bee diversity by asking how resource availability in urban contexts impacts bee health, a determinant of bee diversity.

Bee health is mediated by a multi-host, multi-pathogen system characterized by a broad range of specialist and generalist parasites and pathogens. For example, > 20 RNA viruses infect multiple bee species (Ellis & Munn 2005, Bromenshenk et al. 2010, Dolezal et al. 2016). Bees are also susceptible to parasites. Microsporidian *Nosema* spp. and trypanosomatid *Crithidia* spp. attack *A. mellifera* honey bees and bumble bees in the genus *Bombus* (Paxton et al. 2007, Chen et al. 2008, Plischuk et al. 2009, Otterstatter & Thomson 2008). Lesser-known parasites, such as the neogregarine *Apicystis bombi*, have a cosmopolitan distribution in bumble bees (Ravoet et al. 2014). And larger arthropod parasitoids, such as the phorid fly *Apocephalus borealis*, alter bumble bee and honey bee behavior (Core et al. 2012). Although the transmission mechanism of each parasite and pathogen varies, the importance of flowers as potential transmission hubs is increasingly recognized. Bees rely on flowers for pollen and nectar, but horizontal transmission of parasites and pathogens at flowers may occur via direct bee-to-bee contact or exposure to infected feces, pollen, or nectar (Durrer & Schmid-Hempel 1994, Singh et al. 2010, Graystock et al. 2016). Because multiple pathogens traditionally associated with honey bees have been found in sympatric wild bee populations (Peng et al. 2011, Zhang et al. 2012, Fürst et al. 2014, Ravoet et al. 2014, McMahon et al. 2015), and because both viruses (Singh et al. 2010) and parasites (Graystock et al. 2015) have been found to disperse between species through shared flower visits, flowers may facilitate widespread transmission of pathogens between domesticated and wild bees.

With increased floral provisioning, there can be one of two outcomes for parasite and pathogen infection: dilution or amplification (Becker et al. 2015). Dilution may occur when land-use change increases the quality and quantity of food and habitat resources for a host, resulting in higher immunity or defense against parasites and pathogens. Amplification may occur if increases in resources result in host aggregation, increasing exposure rates and transmission between individuals (Becker et al. 2015). For bees, it is poorly understood how parasite and pathogen interactions respond to changes in resource availability, but there is some evidence that poly-floral diets impart immune benefits in laboratory experiments (Di Pasquale et al. 2013, Mao et al. 2013). However, any immunity advantages conferred by floral diversity may be confounded by increased aggregation and contact rates between infected bees attracted to flowers in a field setting. Resource provisioning may also influence organismal health by mediating the composition of host communities, which may have consequences for disease transmission (LoGuidice et al. 2003, Kilpatrick et al. 2006, Streicker et al. 2013). For bees, the availability of flowers and natural habitat mediates richness and community structure in both natural and agricultural systems (Potts et al. 2003, Kremen et al. 2004). Bee diversity, in turn, may influence parasite richness and abundance. Host richness may promote parasitism if parasites have more hosts to colonize (Rottstock et al. 2014, Kamiya et al. 2014, Johnston et al. 2015, Graystock et al. 2016). However, host richness can also dilute risk if competent hosts are abundant or if transmission is greater within species than between species (Johnson et al. 2013).

Beyond floral provisioning, other features of urban agriculture management may impact disease transmission in bees. Although the role of nesting resources in facilitating transmission is not understood, transmission could occur between infected hosts as bees actively locate nesting sites and forage for nesting materials such as leaves, petals, and sap (MacIvor & Packer 2015). The availability of nesting substrates (such as bare ground and the abundance of pithy stems) also structures bee communities (Potts et al. 2003, Potts et al. 2005). Epidemiology is further complicated by features of the landscape outside of the garden: processes such as urbanization across large scales may indirectly impact bee or pathogen physiology, bee or pathogen behavior, bee community structure, and more (Kilpatrick et al. 2010). Finally, the popular urban practice of honey beekeeping may influence infection in wild bees. For instance, infection of the bumble bee *Bombus vosnesenskii* by *A. borealis* phorid flies in urban gardens increases with honey bee density (Cohen et al. 2017), although the mechanisms are unknown.

Urban garden management may increase or decrease disease risk for bees because the transmission of parasites and pathogens is associated with the availability of floral and nesting resources, bee community diversity, and landscape composition (Fig. 2.1). However, it is important to tease apart the relative contributions of management practices towards parasite and pathogen prevalence. Specifically, we ask 1) Do parasites and pathogens co-occur in individual bees more likely than expected in urban gardens? 2) Which local and landscape factors related to floral and nesting resources correlate with infection by parasites and pathogens? 3) Which bee

community characteristics are associated with infection by parasites and pathogens? 4) Is parasite and pathogen prevalence in wild bees associated with co-occurrence of infection in honey bees? Because resource-mediated dilution and amplification have both been observed in non-arthropod systems in which food was either intentionally or accidentally provided to animals (Miller et al. 2003, Cross et al. 2007, Lane et al. 2011, Jessop et al. 2012, Becker et al. 2015), and because parasites and pathogens vary in their associations with resources, we expected to see both resource-mediated dilution and amplification for bees. Because honey bees are generalist foragers and because they may act as consistent sources of available hosts for parasites if they are managed (possibly exposing wild bees to parasites and pathogens), we also hypothesized that increases in honey bee abundance and co-infection in honey bees is associated with increased parasite and pathogen prevalence in wild bees.

Methods

Characterization of Study Sites

Between two sampling periods in June and July 2015 we examined local and landscape characteristics of 18 urban gardens, ranging in size from 444 m^2 to 15,525 m^2, each separated by 2 km, across three counties (Monterey, Santa Clara, and Santa Cruz) in the California central coast. We measured local habitat characteristics (e.g. vegetation and ground cover) two times within a 20 x 20 m plot placed at the center of each garden. We counted and identified all trees and shrubs and noted the number of individuals in flower. In each plot, we randomly selected four 1 x 1 m plots within

which we identified all herbaceous plants (crops, weeds and ornamentals) to morphospecies, measured height of the tallest non-woody vegetation, counted flowers, and assessed percent ground cover from bare soil, grass, herbaceous plants, leaf litter, rocks, and mulch. In a 100 x 100 m plot surrounding each garden, we counted all trees and quantified percent area with concrete and buildings, mulch, lawn, woody vegetation, weedy or non-woody vegetation, and bare ground. For analysis, values were averaged across the two sampling periods. We also estimated the total garden size. Overall, we measured 20 local habitat variables.

At the landscape scale, we classified land cover types within 2 km buffers surrounding each garden with data from the 2011 National Land Cover Database (NLCD, 30 m resolution) (Homer et al. 2011). We selected 2 km buffers as most bees forage within 2 km from a nesting site (Kremen et al. 2004). We created four land-use categories: 1) natural habitat (deciduous [NLCD number 41], evergreen [42], and mixed forests [43], dwarf scrub [51], shrub/scrub [52], and grassland/herbaceous [71]), 2) open (lawn grass, parks, and golf courses [21]), 3) urban (low [22], medium [23], and high intensity developed land [24]), and 4) agriculture (pasture/hay [81] and cultivated crop [82]). Other land cover types that covered <5% of the total area at each site were not included. We assessed land cover with spatial statistics tools in ArcGIS v. 10.1.

Bee Community Diversity Assessment

We sampled bee community diversity at each site using elevated pan traps and aerial nets across two sampling periods between mid June and early July 2015. We used both methods to attain an accurate inventory of bees in each field site (Grundel 2011). We constructed pan traps by spray painting 400-ml plastic bowls (yellow, white, blue) with Clear Neon brand UV paint and glued each bowl to a PVC coupler. On trapping days, we placed three 1 m tall PVC pipes 5 m apart in a triangle formation within the 20 x 20 m experimental plot in each site, and placed one bowl of each color atop the pipes (Tuell & Isaacs 2009). Pan traps were filled with 300 ml of water and 4 ml of dish soap and placed between 8-9 AM and collected between 3-5 PM. Upon collection, contents were emptied into containers and transported to the lab, and stored in 70% ethanol solution for identification. We sampled bees using aerial netting once per sampling period. We searched for bees for 30 min (not including handling time) at each site between 9:30 AM and 4:30 PM. To identify bees, we primarily relied on dichotomous keys. We identified each bee to genus, and whenever possible, to species (Michener et al. 1994, Ascher & Pickering 2017). For those we were unable to identify to species, we used a morphospecies classification.

Collecting Bees for Parasite and Pathogen Detection

For three sampling periods within a 15-day period between late June and early July, we collected the honey bee *Apis mellifera* (Hymenoptera: Apidae) and the yellow-faced bumble bee *Bombus vosnesenskii* (Hymenoptera: Apidae) from each site for parasite and pathogen detection. We chose these two species because both are

commonly found in our sites, they exhibit different social life history strategies, and they host an overlapping suite of parasites and pathogens (McMahon et al. 2015). The honey bee is commonly present in our field sites as an introduced, domesticated species (although only four sites managed honey bee hives), whereas the yellow-faced bumble bee is a native wild visitor. At each sampling period we used aerial nets to collect bees for 30 minutes. Each bee was placed into a sterile 2 ml vial and immediately stored in dry ice. We sterilized gloves and nets between sampling sites with bleach then ethanol. Bees were transported to the lab and into -80 °C cold storage.

DNA and RNA Extraction

We simultaneously extracted DNA and positive-strand RNA from each specimen by creating a modified protocol combining procedures from the Qiagen DNeasy blood and tissue extraction kit and Qiagen QIAamp Viral RNA Mini Kit. We homogenized each bee in cold PBS with bead-beating for 6 min at 30 Hz with sterile stainless steel beads and 0.1 mm glass beads in a Qiagen Tissue Lyser II (Qiagen, Hilden, Germany). We centrifuged homogenate briefly, then transferred 180 µl into a microcentrifuge tube for DNA extraction. We centrifuged the remaining homogenate at1500 g for 10 min, then removed 140 µl of solution for RNA extraction. We hydrolyzed DNA extract with 20 µl Proteinase K in 200 µl Buffer AL without added ethanol. After 12 h. incubation at 56 °C, we followed standard spin column protocols with a single final elution. We pulse vortexed RNA extract with 560 µl of Buffer

RNA-AVE/AVL to isolate RNA; after incubation at room temperature for 10 min, we followed standard spin column protocols. We used a NanoDrop instrument to analyze nucleic acid concentration was analyzed (Thermo Fisher Scientific, Waltham, MA).

Species Identification

Because *B. vosnesenskii* is nearly morphologically identical to the less-common *B. caliginosus*, we confirmed the identity of all *Bombus* species by sequencing the protein-coding elongation factor -1 alpha gene using primer pair F2-ForH/F2-RevH2 (Hines et al. 2006, Kawakita et al. 2003, 1). The forward strand of each DNA product was sequenced using Sanger Sequencing (Applied Biosystems 3730xl DNA Analyzer, Retrogen), aligned by eye in Mesquite 3.04 (Maddison & Maddison 2015), then queried against the National Center for Biotechnology Information (NCBI) nucleotide data with BLAST.

Parasite and Pathogen Detection

We tested each bee for a suite of parasites and pathogens that vary by taxonomic classification, symptoms, and transmission mechanism (Table 2.2). We tested 492 honey bee specimens and 254 bumble bee specimens for parasites. We then tested a subset of each of these specimens for infection by RNA viruses, 292 honey bees and 241 bumble bees. To test for parasites, we screened DNA for the presence of *Apicystis*, *Crithidia*, and *A. borealis* using parasite specific primers and conditions for genus-level identification (Table 2.1). Products were run alongside a

standard ladder on a 1% agarose gel stained with GelRed to confirm amplicon size. Each assay included a negative and positive control.

To screen for viruses, we used a multiplex reverse transcription PCR protocol, Multiplex Ligation-dependent Probe Amplification (MLPA), developed by DeSmet et al. (2012) to simultaneously detect Chronic Bee Paralysis Virus (CBPV), Deformed Wing Virus (DWV) & relatives, Acute Bee Paralysis Virus (ABPV) complex, Black Queen Cell Virus (BQCV), Slow Bee Paralysis Virus (SBPV), and Sacbrood Virus (SBV) (Table 2.2) and a positive control gene β-actin with the use of one primer set. The protocol uses a probe consisting to two oligonucleotides which recognize adjacent target sites on the DNA that must be ligated for amplification. Unique probe lengths allow for a high resolution of multiple targets. We used an MLPA kit (RT EK5, unlabeled primers) from MRC-Holland (Amsterdam, Netherlands). Amplicons were resolved at the University of California Riverside Institute for Integrative Genome Biology using a fragment analyzer (Applied Biosystems 3130XL Genetic Analyzer, Foster City, CA) with a DNF-905 reagent kit (Advanced Analytical Technologies Inc, Ankeny, IA) to visualize fragments between 1-500bp. We used a detection limit of 75 RFU (relative fluorescent units) for interpretation of fragments to obtain yes/no prevalence data for each virus.

Data Analysis

We first examined co-occurrence of parasites and pathogens in honey bee (n=292) and bumble bee (n=241) individuals using the 'cooccur' package in R

(Veech 2013, Griffith et al. 2016). We separately calculated co-occurrence with presence/absence data for parasites (*Apicystis*, *Crithidia, A. borealis)* and pathogens (CBPV, DWV & relatives, ABPV complex, BQCV, SPBV, SBV).

We then examined the relative contribution of garden management and bee community characteristics towards parasite and pathogen prevalence. Because many local, landscape, and bee community characteristics that we measured may be correlated, we performed a variable selection process. We divided most variables into four groups (floral resources, nesting resources, landscape composition, and bee community diversity) and ran Pearson's correlations within groups to identify correlated ($P<0.05$) variables within groups. We selected variables that were correlated with the largest number of other variables in that group for subsequent analysis (Table 2.3). In addition, we included honey bee infection prevalence when examining bumble bee infection, and vice versa. In all, we selected four floral resource variables, one nesting resource variable, one landscape variable, three bee community variables, and a co-infection variable to include in subsequent analysis (Table 2.3). We log transformed variables that did not meet conditions of normality. We ran an additional test to identify whether any of the ten selected variables were collinear by calculating a variance inflation factor (VIF) for each predictor using the 'car' package in R (Fox et al. 2012). We found that each variable met our VIF cutoff score of 4.

We used generalized linear models (GLMs) with the glm function in R to examine relationships between prevalence of each parasite and pathogen in honey

bees and bumble bees and the ten variables describing floral resources, nesting resources, landscape cover, bee community diversity, and co-infection in honey bees or bumble bees. We modeled a two-vector response variable (infected individuals, not-infected individuals) using the cbind function to maintain information about the sample size and required a binomial error structure. One method to test combinations of variables is the 'glmulti' package (Calcagno & de Mazancourt 2010), but it does not support a binomial distribution. We therefore used this package to compare GLM models using a guassian error structure, with percentage of infected individuals at a site as our responsive variables. We used the output to determine the top models within 2 AICc points. This method informed us which combinations of variables to compare when subsequently modeling a two-vector response variable bound by cbind and a binomial error structure. These models were then compared by delta AIC and AIC weight. To determine the goodness-of-fit of the best models, we calculated a pseudo-R^2 value as [(null deviance - residual deviance)/ null deviance] (Dobson & Barnett 2008). We analyzed all data in R v.3.2.3 (R Core Team 2015).

Results

Co-occurrence of parasites and pathogens

The number of specimens tested and the percentage of individuals found with each parasite and pathogen is reported in Table 2.4. For bee individuals, the majority of parasites (*Crithidia*, *Apicystis*, and *A. borealis*) occurred singly (47.42% of honey bee and 40.94% of bumble bee individuals had a single infection), with co-occurrence

of zero, two, and three parasites being detected in, respectively, 29.76%, 21.83% and 0.99% of honey bee individuals and in 17.72%, 30.35% and 11.02% in bumble bees. The most prevalent parasite in the garden sites was *Crithidia* in honey bees (46.00%) and *Apicystis* in bumble bees (47.49%).

For honey bees, the majority of individuals presented with a triple infection of RNA viruses (28.67%), with no infection occurring in 11.26% of honey bees, single infection occurring in 20.48%, two viruses in 23.89%, four viruses in 13.99%, five viruses in 1.02%, and six viruses in 0.68% of bees. For bumble bees, the majority of individuals presented with a double infection of RNA viruses (28.93%), with no infection occurring in 5.79%, single infection occurring in 14.88%, three viruses in 25.20%, four viruses in 19.83%, five viruses in 4.55%, and six viruses in 0.82% of bees. The most prevalent RNA virus was SBPV in honey bees (59.54 %) and CBPV in bumble bees (74.98%).

In a probabilistic model of co-occurrence examining interactions between parasite and pathogen pairs, interactions were not evenly distributed between parasites and pathogens, but clustered around a few parasites and pathogens (Fig. 2.2). For both honey bees and bumble bees, SBPV had the highest number of significant ($p<0.05$), positive pair associations with other parasites and pathogens (50% of pairs and 62.50% of pairs, respectively). In bumble bees, DWV had zero associations, with 100% of pairings due to random associations, but for honey bees every parasite and pathogen had at least one negative or one positive association with another parasite and pathogen (Fig. 2.2).

Local, landscape, and bee community drivers of parasite prevalence

Parasite prevalence was influenced by nesting and floral resource availability, landscape features, and the prevalence of co-infection in either honey bees or bumble bees. For honey bees, the prevalence of *Crithidia* was predicted by natural cover within 2 km ($z=-3.571$, $P<0.001$, Fig. 2.3c). The model that predicted *Apicystis* included the abundance of trees and shrubs in flower ($z=2.259$, $P=0.024$, Fig. 2.3b) and co-infection in bumble bees ($z=5.345$, $P<0.001$, Fig. 2.3a). The model that predicted *A. borealis* prevalence included the abundance of flowers ($z=2.457$, $P=0.014$, Fig. 2.4b), natural cover within 2 km ($z=3.09$, $P=0.002$, Fig. 2.4c) and co-infection in bumble bees ($z=2.763$, $P=0.006$, Fig. 2.4a).

For bumble bees, *Crithidia* prevalence was not predicted by any significant variables. The model that predicted *Apicystis* included co-infection in honey bees ($z=6.423$, $P=<0.001$, Fig. 2.3d). The model that predicted *A. borealis* prevalence included bare soil ($z=-3.251$, $P<0.001$, Fig. 2.4f), honey bee abundance ($z=3.102$, $P=0.002$, Fig. 2.4e), and co-infection in honey bees ($z=2.640$, $P=0.008$, Fig. 2.4d).

Local, landscape, and bee community drivers of virus prevalence

Pathogen prevalence was influenced by nesting and floral resource availability, bee richness, landscape features, and co-infection of each parasite and pathogen (Table 5a, Table 5b). For honey bees, DWV was predicted by bare soil ($z=2.141$, $P=0.032$, Fig. 2.5a), ABPV complex by bee richness ($z=2.78$, $P=<0.01$, Fig. 2.5a) and co-infection in bumble bees ($z=-2.403$, $P=<0.05$, Fig. 2.5a), BQCV by

natural cover within 2 km (z=2.945, P=0.003, Fig. 2.5c), and CBPV by honey bee abundance (z=-4.264, P<0.001, Fig. 2.5e). SBPV and SBV were not significantly predicted by any of the variables.

For bumble bees, DWV was predicted by garden size (z=2.077, P=0.038, Fig. 2.6d), ABPV complex by the abundance of trees and shrubs in flower (z=1.988, P=0.047, Fig. 2.6a) and bumble bee abundance (z=2.078, P=0.038, Fig. 2.6b), BQCV by bare soil (z=-2.143, P=0.032, Fig. 2.6c), and SBV by bumble bee abundance (z=2.967, P=0.003, Fig. 2.6e). SBPV was predicted by the richness of crops, weeds, and ornamentals (z=3.234, P=0.001, Fig. 2.6j), the abundance of trees and shrubs in flower (z=2.71, P=0.007, Fig. 2.6f), natural cover within 2 km (z=4.391, P<0.001, Fig. 2.6i), honey bee abundance (z=-3.627, P<0.001, Fig. 2.6h), and bumble bee abundance (z=4.13, P<0.001, Fig. 2.6g). CBPV was not significantly predicted by any of the variables.

Discussion

Floral resources amplify and nesting resources dilute disease risk

When floral resources influenced disease outcome, higher floral abundance related to higher parasite and pathogen prevalence. This is the first reported study of resource-mediated disease amplification in bees. Further, while previous wildlife-focused studies examined the role of food resources in resource provisioning, this study examines the role of nesting resources in epidemiology. We found that bare soil, a possible indication of available below-ground nesting availability for bumble

bees, negatively predicted the prevalence of some parasites and pathogens for these bees. The availability of nesting sites may mitigate disease risk for bumble bees if nests indirectly confer an immune benefit or if increases in bare soil result in spatially dispersed nests and thus lower contact between infected bees. For honey bees, the impact of bare soil was different. Nesting resources only emerged as an important predictor for the prevalence of DWV complex in honey bees, and was a positive predictor of parasitism. We measured bare soil as a proxy for available nesting sites, but honey bees nest in above ground site; limitations to our methodology may therefore explain why we found this outcome. For honey bees, bare soil may positively predict infection if bare soil indicates a lack of above ground cavities for honey bee hives, though we did not measure above ground nesting availability. It is also important to note that some parasites and pathogens replicate through transmission mechanisms not associated with resource foraging and sharing (Imhoof & Schmid-Hempel 1999, Shen et al. 2005. DWV is one such virus -- it is transmitted through pollen and the oral-fecal route, but also through *Varroa* mite, transovarian, and semen transmission (de Miranda et al. 2013). Transmission of DWV therefore occurs within the honey bee colony, suggesting a means by which nesting might positively predict DWV prevalence in honey bees. Nesting resources are generally understudied in the disease literature because it is difficult to locate and measure nesting sites, but because the availability of nesting sites may mitigate or amplify disease risk, we believe this is an important direction for future study.

Although local floral resources were significant positive drivers of parasite and pathogen prevalence, they were more likely to be important for bumble bees than honey bees. Bumble bees may be more sensitive to variation in floral resources because they have recorded foraging distances of 0.8 - 2.8 km (Jha & Kremen 2013), whereas honey bees are known to forage across long distances up to 20 km. This may explain why natural landscape cover was significant for three parasites and pathogens of honey bees, but for only one pathogen of bumble bees. The impact of natural cover was not even across parasites and pathogens, possibly because natural habitat may include both floral and nesting resources. If floral resources amplify transmission but nesting resources, overall, dilute transmission, the relationship between the amount of natural cover around a garden and parasite and pathogen prevalence may depend on the composition of that natural habitat. Regardless of the amount of floral resources in urban gardens or around them, we also cannot assume that the impact of floral resources is even across flower types. Generally, our garden sites include domesticated perennial fruit trees and ornamentals, as well as annual crops, ornamentals, and weeds, whereas natural habitat around each site includes vegetation such as grassland, shrubs, and forest. Flowers from natural habitats may differ from domesticated flowers in appearance, phenology and more, and variation in flower traits has been identified as a mechanism influencing disease transmission (McArt 2014).

Bee species share parasites and pathogens, but the impact of bee community composition is mixed

This study corroborates that bee species share parasites and pathogens (Goulson 2009, Cornman et al. 2012, Evison et al. 2012, Ravoet et al. 2014, Gamboa et al. 2015, McMahon et al. 2015). We found that co-infection in honey bees was a significant positive driver of infection prevalence in bumble bees. However, co-infection in honey bees was only important for infection prevalence in bumble bees for *some* parasites and pathogens. We found that the availability of resources, landscape composition, and the diversity of the bee community sometimes also emerged as more significant factors driving parasite and pathogen prevalence in bumble bees. Often these factors were the only predictors of prevalence. Furthermore, ABPV prevalence in bumble bees negatively predicted ABPV prevalence in honey bees, complicating our findings. Because ABPV in bumble bees is itself in part predicted by increased bumble bee host abundance, this finding may be explained if bumble bees are the preferential host of ABPV. There are additional unknown, indirect relationships mediating the link between host density and virus epidemiology for multi-host pathogens.

We expected that bee richness would be an important predictive variable. While increased bee richness possibly translates into a possible increase in alternative hosts for parasites and pathogens, the presence of additional species within a garden may also dilute the likelihood of disease transmission. We found that bee richness was only important for modeling the prevalence of viruses in the ABPV complex in

honey bees. In addition, we found mixed and uneven impacts of host density on parasite and pathogen prevalence. One of the limitations to understanding the role of bee community diversity and host abundance is that few studies have observed how often, when, and where inter-species interactions occur. Future studies observing how bee species interact at flower sites or utilize overlapping resources may shed light on some of the dynamics of disease epidemiology.

Conclusion

We found very high infection rates for most parasites and pathogens in both *Apis mellifera* and *Bombus vosnesenskii*. While *Apicystis* has previously been considered a low infection rate parasite of *Bombus*, it was recently reported in 30-50% of sampled honey bee and bumble bee colonies (Graystock 2013, Graystock. 2014). Our study found similarly high rates at 52.76% prevalence in bumble bees and 41.77% in honey bees. Differences in infection rates may reflect habitat or bee density differences. In another study (e.g. Plischuk et al. 2011), bees were collected in agricultural, natural, or lab environments, not urban environments. Environmental context can strongly impact disease dynamics, for example by altering host or parasite condition. Furthermore, condition-dependent pathogens can appear asymptomatic under good, resource abundant conditions, and negative impacts on host fitness may only become apparent when under stressful or resource-limited conditions (Manley et al. 2017). It is important to note that testing positive for parasite or pathogen presence does not necessarily indicate that a parasite or pathogen

is replicating in its host. However, even individuals passively carrying a parasite or pathogen might still be infectious to others (Graystock et al. 2013).

The interactions between bees and their parasites and pathogens are complex because characteristics of host and parasite and pathogen communities are tightly connected: resource supply to hosts may influence parasite or pathogen richness and abundance. For example, resource availability may increase host biomass or makes host tissue more nutritious to parasites, thus leading to increases in parasite abundance. Indirect impacts may thus be mechanistic drivers of prevalence rates. For example, bees and their insect parasitoids often have overlapping resource requirements for pollen and nectar (e.g. phorid fly adults and bees both consume nectar). Understanding landscape epidemiology therefore necessitates careful evaluation of complex, non-independent relationships among bees and their parasites. One promising method for quantifying these pathways is the ecosystem multifunctionality framework (Lefcheck et al. 2015, Dooley et al. 2015). This framework describes how biodiversity alters the relationship among non-independent functions, and has been applied to understand how insect and microbial parasite diversity and abundance respond to host diversity and resource availability in plant disease systems (Halliday et al. 2017). Future studies may consider employing multivariate statistical approaches stemming from the ecosystem multifunctionality framework to model resource-mediated disease risk in bees.

Because many pollinator species have undergone range contractions and extinctions over recent decades (Kosior et al. 2007, Goulson et al. 2008, Williams &

Osborne 2009), understanding how resource availability impacts disease transmission is important for conservation efforts. Intentional and accidental resource provisioning for bees in urban gardens occurs when gardeners plant flowers and alter groundcover characteristics. In examining the determinants of bee decline, it is important to consider how provisioning can alter parasite and pathogen dynamics for wild and domesticated bees.

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Authors' contributions

HC acquired funding for the research, led study design, fieldwork, lab work, and coordinated manuscript writing and publication. Co-author Kaleigh Russell participated in lab work and manuscript writing. Co-author Quinn McFrederick contributed to field research design, provided physical and financial access to laboratory equipment, supervised laboratory methods, and contributed to the manuscript. Co-author Stacy Philpott contributed to field research design, fieldwork logistics, data analysis, and manuscript writing.

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Table 2.1 PCR mixes and conditions for identification of parasites and pathogens

Primers	Primer Name	dNTP (µl)	Taq (µl)	10x Buffer (µl)	Primer F (µl)	Primer R (µl)	Template (µl)	Total vol. (µl)	1 Denaturing Min | Temp	2 Replication Sec | Temp	3 Elongation Min | Temp	Amplicon size (bp)
Bombus spp. *EF-1α* (Hines et al. 2006; Kawakita et al. 2003)	F2-ForH, F2-RevH2	0.2	0.05	1	0.2	0.2	1	10	3 | 94	36x 60 | 94 60 | 54.5 60 | 72	5 | 72	24
Apicystis spp. (Meeus et al. 2010)	NeoF, NeoR	0.2	0.05	1	0.2	0.2	1	10	2 | 95	35x 30 | 94 30 | 60.7 45 | 72	3 | 72	850
Crithidia spp. (Meeus et al. 2010)	SEF, SER	0.2	0.05	1	0.2	0.2	1	10	2.5 | 95	35x 30 | 94 30 | 56.5 52 | 72	4 | 72	417
Apocephalus borealis (Core et al. 2012; Runckel et al. 2011)	PhoridrRNA-1F, PhoridrRNA-1R	0.2	0.05	1	0.2	0.2	1	10	3 | 95	35x 30 | 95 30 | 56.5 52 | 72	5 | 72	500

Table 2.2 Natural history and transmission information for all parasites and pathogens selected for screening.

Parasite	**Type**	**Transmission**	**Symptoms (*Bombus spp*.)**	**Symptoms (*Apis mellifera*)**
Crithidia spp.	Parasite	oral-fecal, transovarian (Imhoof & Schmid-Hempel 2014)	impaired offspring production and colony initiation, reduced queen survival, lower foraging efficiency (Brown et al. 2003, Gegear et al. 2006)	inability to determine if flowers have nectar (COLOSS)
Apicystis spp.	Parasite	unknown	disruption of adipose tissue, impaired colony initiation, community conflict, premature mortality (Schmid-Hempel 2001, Rutrecht and Brown 2008)	unknown
Apocephalus borealis (phorid fly)	Parasitoid	unknown	premature mortality (Otterstater et al. 2002)	disorientation, hive abandonment, premature mortality (Core et al. 2012)
DWV: Deformed Wing Virus	RNA Virus	oral-fecal, pollen, transovarian, semen, *Varroa* (de Miranda et al. 2013)	wing abdormalities (Genersch et al. 2006)	wing abdormalities impaired cognitive function (COLOSS)
ABPV: Acute bee paralysis virus	RNA Virus	oral-fecal, transovarian, semen, *Varroa* (de Miranda et al. 2013)	impaired offspring production and colony initiation (Meeus et al. 2014)	mostly symptomless, can be lethal at individual and colony level (COLOSS)
BQCV: Black queen cell virus	RNA Virus	oral-fecal, pollen, *Varroa* (de Miranda et al. 2013)	unknown	blackened cell walls, dead pro-pupae (COLOSS)
SBPV: Slow bee paralysis virus	RNA Virus	oral-fecal, *Varroa* (de Miranda et al. 2013)	premature mortality (Manley & Wilfert 2017)	paralysis front legs (COLOSS)
SBV: Sacbrood virus	RNA Virus	oral-fecal, pollen, food, transovarian (Shen et al. 2005)	unknown	kills larvae, changes foraging behavior in adults (COLOSS)
CBPV: Chronic Bee Paralysis Virus	RNA Virus	oral-fecal, contact (Ribière et al. 2007)	unknown	abnormal trembling of wings and body, loss of hair, dysentery, paralysis, premature mortality (Ribière et al. 2007)

Table 2.3 Results of Pearson's Correlations showing groups of explanatory variables, variables selected for GLM analysis, and variables correlated ($P<0.05$) with selected variables. Variables reflect an averaged value per site across 2 sampling periods.

Group	**Selected Variables**	**Correlated Variables**	Correlation coefficient	Direction of correlation
Floral Resources	Abundance of Perennials in Flower	Richness of Perennials	0.889**	+
	Richness of Crops, Weeds, and Ornamentals	NA		
	Abundance Annual Flowers	NA		
	Size	Age	0.539*	+
Nesting Resources	Bare Soil (%)	Leaf Litter (%)	0.471*	-
		Mulch & Straw Cover (%)	0.853**	-
Landscape Characteristics	Natural Cover (2km)	Open Cover (2km)	0.749**	+
		Urban Cover (2km)	0.911**	-
Bee Community	Bee Richness	NA		
	A. mellifera Abundance	Bee Abundance	.622**	+
	B.vosnesenskii Abundance	Bee Abundance	.531*	+

Table 2.4 Infection prevalence rates for each parasite and pathogen[§].

	A. mellifera				*B. vosnesenskii*			
	Infected, All Sites	All Sites	Infected, Avg/Site	SE (+/-)	Infected, All Sites	All Sites	Infected, Avg/Site	SE (+/-)
Crithidia spp.	224	499	46.00	4.31	124	254	51.97	6.04
Apicystis spp.	199	499	41.77	5.46	134	254	47.49	6.22
A. borealis	50	499	9.74	2.70	84	254	22.55	6.03
DWV complex	30	292	10.40	2.46	9	241	4.61	2.92
ABPV complex	108	292	36.07	3.57	87	241	25.71	4.55
BQCV	62	292	21.36	2.76	21	241	5.09	1.76
SBPV	177	292	59.54	5.22	124	241	37.98	7.50
SBV	121	292	41.24	2.40	183	241	71.32	6.00
CBPV	148	292	52.54	4.01	194	242	74.98	4.57

[§]Abbreviations for viruses are listed in Table 2.2

Table 2.5a Results of GLM model selection for *A. mellifera*. Table shows significant variables from the best model selected for each parasite and pathogen. (Signif. codes: '***' 0.001, '**' 0.01, '*' 0.05, ' ' 1)

	A. mellifera												
	Nesting Resources		Floral Resources		Landscape		Bee Diversity		Co-Infection		AIC	pseudo-R^2	df
Crithidia spp.					Natural (2km)***	-					106.52	0.33	16
Apicystis spp.			Abundance Trees & Shrubs in Flower*	+					*B. vosnesenskii* Infection***	+	113.93	0.42	16
A. borealis			Abundance Flowers*	+	Natural (2km)**	+			*B. vosnesenskii* Infection**	+	55.818	0.72	16
DWV complex	Bare Soil (%) *	+									59.985	0.22	16
ABPV complex							Bee Richness**	+	*B. vosnesenskii* Infection*	-	65.993	0.69	16
BQCV					Natural (2km)**	+					64.794	0.45	16
SBPV													
SBV													
CBPV							*A. mellifera* Abundance ***	-			72.167	0.65	16

Table 2.5b. Results of GLM model selection for *B. vosnesenskii*. Table shows significant variables from the best model selected for each parasite and pathogen. (Signif. codes: '***' 0.001, '**' 0.01, '*' 0.05, ' ' 1)

	B. vosnesenskii												
	Nesting Resources		Floral Resources		Landscape		Bee Diversity		Co-Infection		AIC	pseudo-R^2	df
Crithidia spp.													
Apicystis spp.									*A. mellifera* Infection***	+	64.314	0.78	16
A. borealis	Bare Soil (%) ***	-					*A. mellifera* Abundance**	+	*A. mellifera* Infection**	+	62.673	0.78	16
DWV complex			Size*	+							26.776	0.43	16
ABPV complex			Abundance Perennials in Flower*	+			*B. vosnesenskii* Abundance*	+			54.577	0.66	16
BQCV	Bare Soil (%) *	-									37.714	0.33	16
SBPV			Richness Crops, Weeds, and Ornamentals **	+	Natural (2km)***	+	*A. mellifera* Abundance***	-			59.971	0.72	16
			Abundance Perennials in Flower**	+			*B. vosnesenskii* Abundance***	+					
SBV							*B. vosnesenskii* Abundance**	+			68.372	0.31	16
CBPV													

Figure 2.1 The landscape epidemiology of flower-vectored parasites and pathogens. To simplify the representation of this complex system, both indirect and direct relationships are represented by a solid line, though it should be noted that most interactions may occur through both direct and indirect mechanisms.

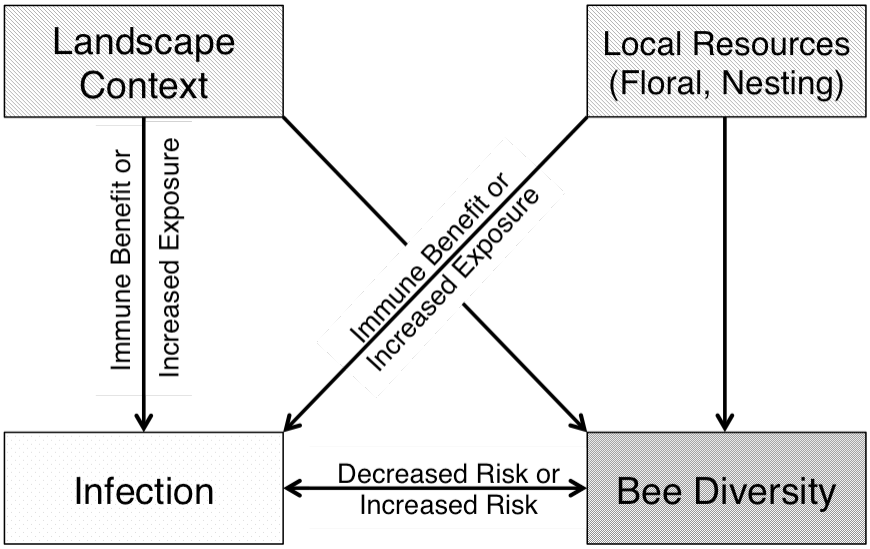


Figure 2.2 Co-occurrence of infective species within *A. mellifera* (left) and *B. vosnesenkii* hosts (right). The heat map indicates positive and negative species associations determined by a probabilistic co-occurrence model. Positive relationships indicate species pairs that could co-occur more than what is expected. Negative relationships indicate that those species could co-occur less than what is expected. Numbers indicating parasites and pathogens are positioned to indicate the columns and rows that represent their pairwise associations with other parasite and pathogen species. If a parasite or pathogen did not have any positive or negative associations, it was excluded from the map.

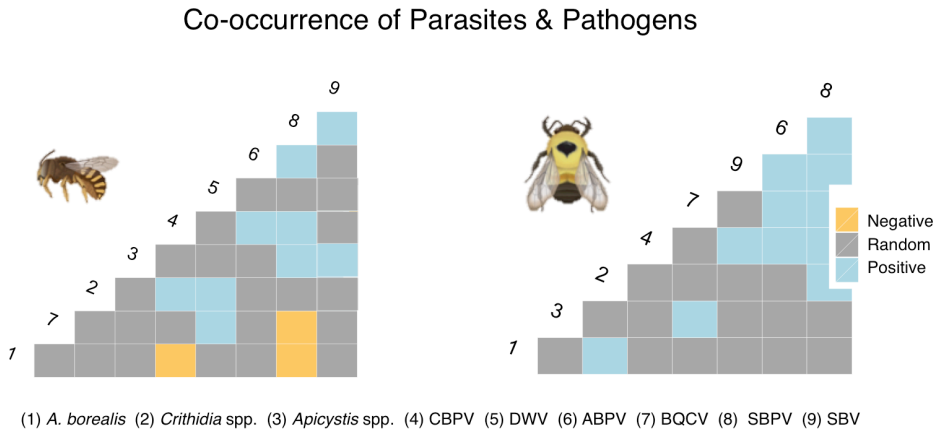


Figure 2.3 Local, landscape, and bee community drivers of prevalence of *Apicystis* spp. (a, b, d) and *Crithidia* spp. parasites (c) detected in *Apis mellifera* (a, b, c) and *Bombus vosnesenskii* (d*)* bees collected from urban gardens in the California central coast. (Signif. codes: '***' 0.001, '**' 0.01, '*' 0.05).

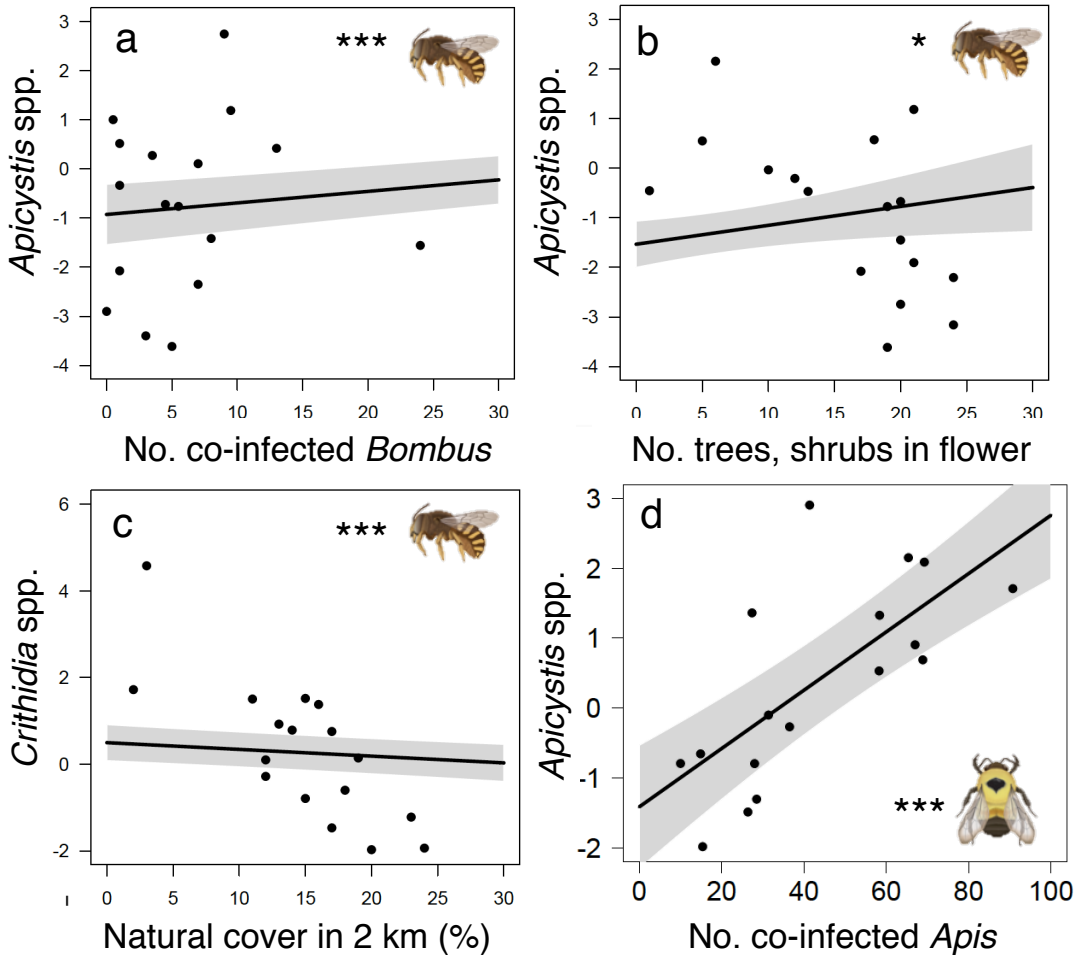


Figure 2.4 Local, landscape, and bee community drivers of prevalence of the phorid fly *Apocephalus borealis* detected in *Apis mellifera* (a, b, c) and *Bombus vosnesenskii* (d, e, f) bees collected from urban gardens in the California central coast. (Signif. codes: '***' 0.001, '**' 0.01, '*' 0.05)

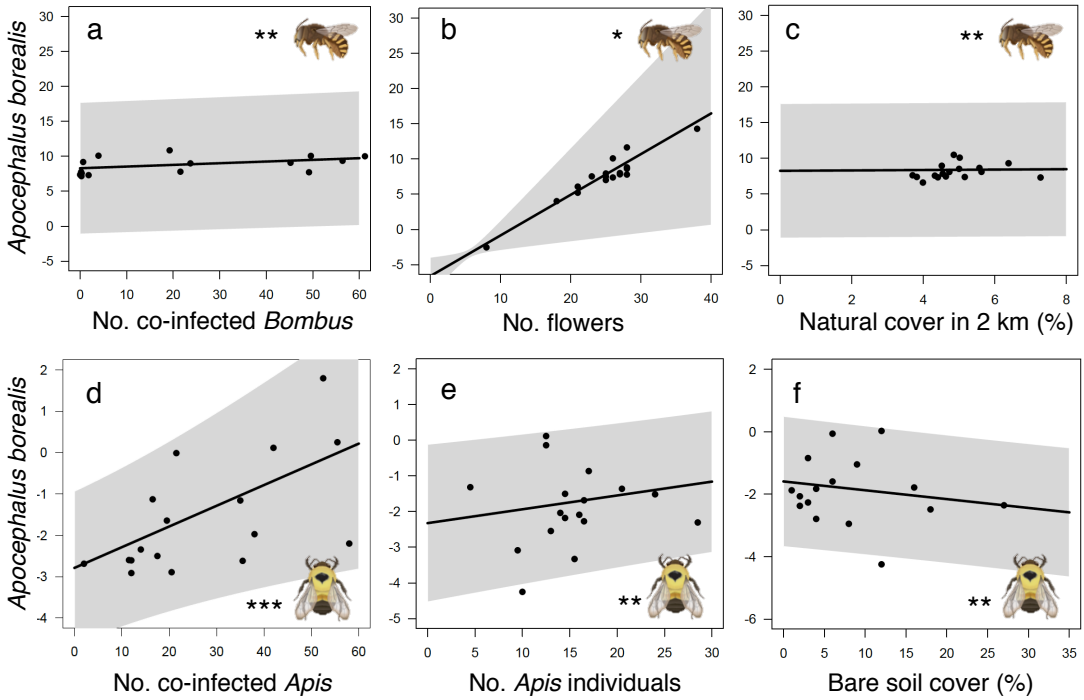


Figure 2.5 Local, landscape, and bee community drivers of prevalence of viruses in *Apis mellifera* collected from urban gardens in the California central coast. For virus abbreviations, see Fig. 2. (Signif. codes: '***' 0.001, '**' 0.01, '*' 0.05)

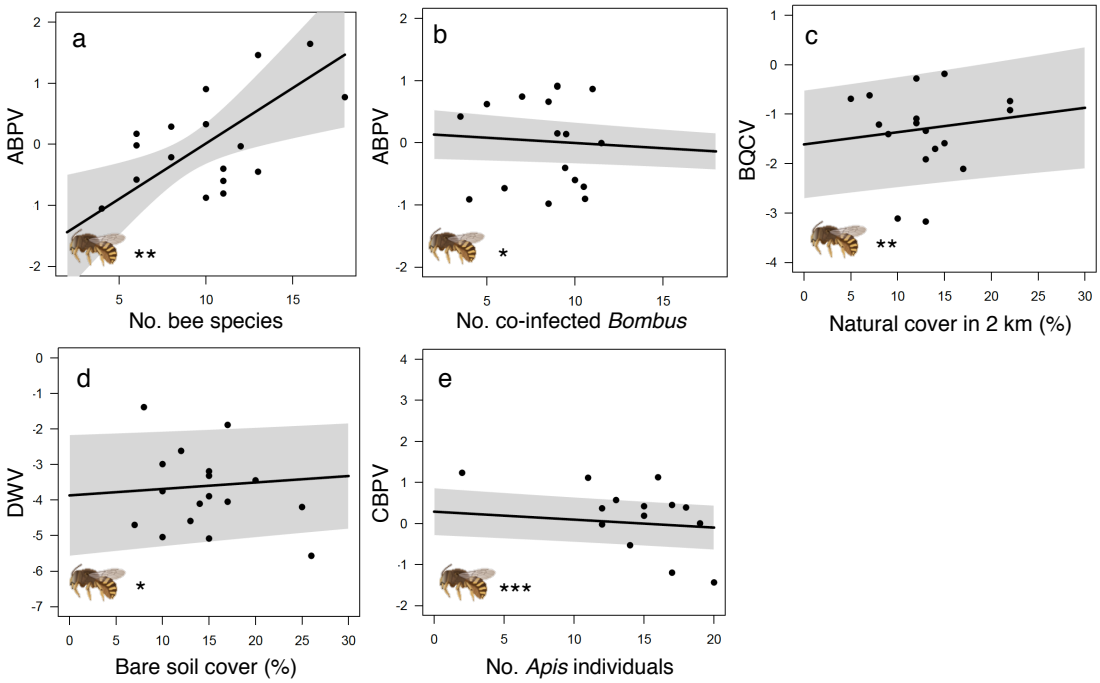
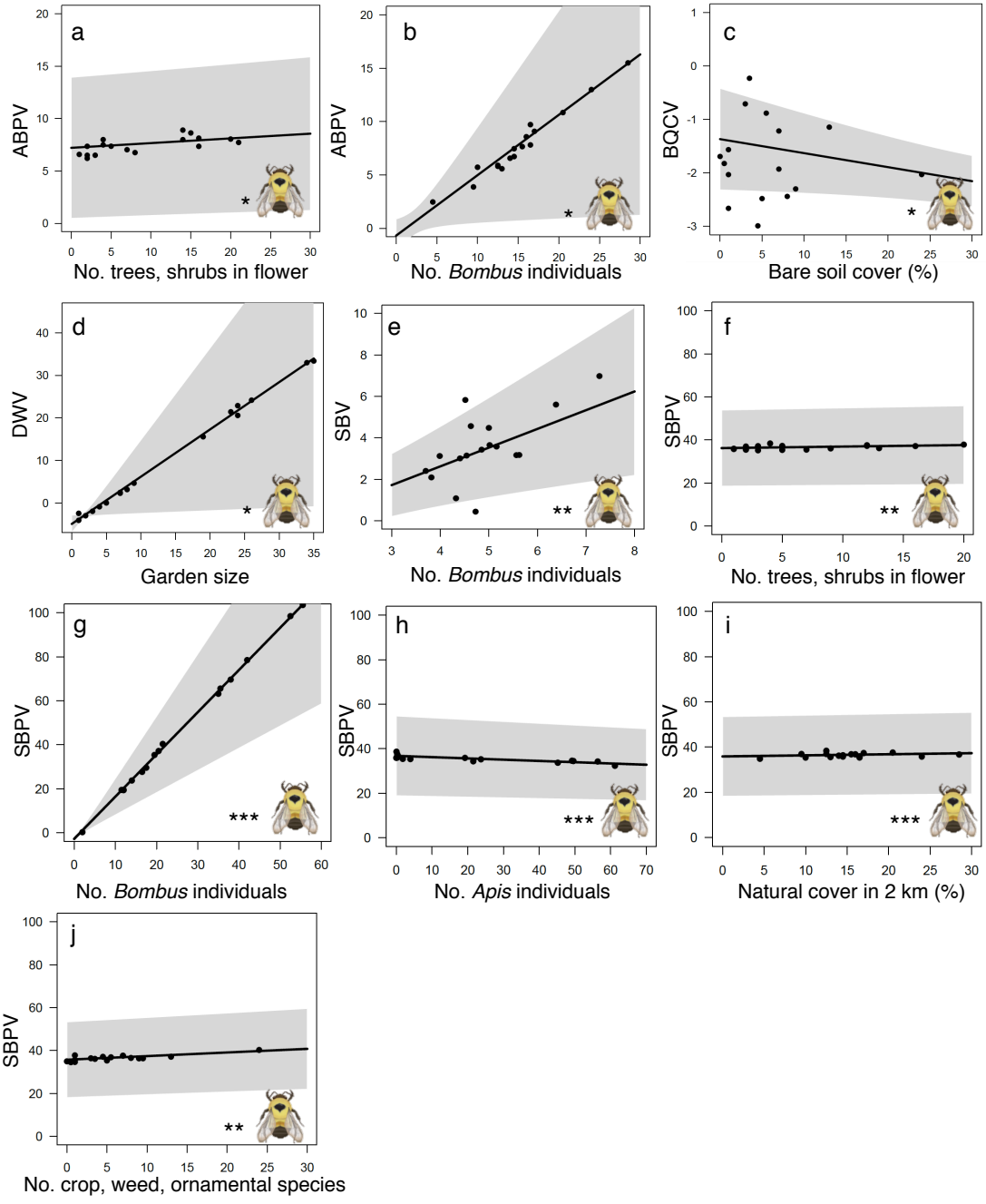


Figure 2.6 Local, landscape, and bee community drivers of prevalence of viruses in *Bombus vosnesenskii* collected from urban gardens in the California central coast. For virus abbreviations, see Fig. 2. (Signif. codes: '***' 0.001, '**' 0.01, '*' 0.05).



CHAPTER 3: Environmental drivers of microbiome composition in the Blue Orchard Bee, *Osmia lignaria*

Abstract

Wild bees encounter and collect environmental microbes whilst foraging. While environmental context affects bee diversity, little is known about it how affects the wild bee microbiome. We used field experiments in 17 urban gardens to examine whether and how local and landscape features influence the whole-body microbiome of the Blue Orchard Bee, *Osmia lignaria*. We found that environmental features - specifically natural habitat in the landscape, floral resources, and bee richness – influence differences in microbiome composition between bee individuals. We also found that environmental features were associated with the abundance of bacterial groups important for bee health, such as *Lactobacillus* OTUs. Our study highlights complex interactions between environment context, bee diversity, and the bee-associated microbes.

Introduction

An insect hosts a collection of microorganisms, called the microbiome. The microbiome can impact host fitness through impacts to nutrition, growth rate regulation and stress tolerance, and protection against parasites and pathogens (Dillon & Dillon 2004, Douglas 2009, Ferrari et al. 2004, Henry et al. 2015, Ruokolainen et al. 2016). While the microbiome is considered an extended immune phenotype, it is

not known how ecological processes shape and change the microbiome (Engel et al. 2016). Insects acquire microbes through vertical transmission, but also through horizontal transmission, from the environment and social interactions (Gibson & Hunter 2010, Mason & Raffa 2014). For example, the insect microbiome may be influenced by available diet (Broderick et al. 2004, Lundgren & Lehman 2010, Mason & Raffa 2014, Wang et al. 2011) and the specific geographical location where the insect host is found (Adams et al. 2010, Coon et al. 2016, Toju & Fukatsu 2011, Yun et al. 2014). While the impact of habitat context on the insect microbiome has been studied for predatory insects that rely on arthropod prey as food resources (Tiede et al. 2017), systematic studies on the effect of environmental context on the solitary bee microbiome are lacking.

For solitary bees, the ways in which environmental context impact the microbiome may be especially important because bee decline is attributed, in part, to environmental changes such as loss of floral resources and nesting habitat (Potts et al. 2010, Brown & Paxton 2009, Cameron et al. 2011). Multiple qualitative syntheses suggest that environmental changes (such as agricultural intensification and habitat fragmentation) at local and landscape-level scales have population impacts for bees (e.g. Kennedy et al. 2013, Ricketts et al. 2008; Winfree et al. 2009, Klein et al. 2007), likely through changes to floral and nesting resources (Kremen et al. 2007). The availability of resources may also be important for the microbial associates of bees. For honey bees and bumble bees, a distinctive hindgut microbiome is obtained by direct transmission between members of the same species (Martinson et al. 2011,

Koch & Schmid-Hempel 2011a), but for most wild and solitary bees, microbes are likely acquired from the environment. For example, halictid and megachilid bees acquire *Lactobacillus* bacteria from flowers (McFrederick et al. 2012, McFrederick et al. 2017). Solitary bees may also acquire microbes through contact with feces, either from flowers or nesting materials previously visited by other bees or through direct interactions with other bees while foraging for food and nesting materials. Thus, resource availability and bee diversity at local and landscape scales may influence microbiome acquistion.

In addition to habitat loss, parasites and pathogens also contribute to bee population declines (Goulson et al. 2015, Brown & Paxton 2009, Cameron et al. 2011). But insect-microbe associations can influence the outcome of insect infections by viruses, bacteria, and parasites (Dillon et al. 2005, Jaenike et al. 2010). For example, the ubiquitous endosymbiont *Wolbachia pipientis* is associated with fitness benefits to *Drosophilia melanogaster* flies infected by RNA viruses (Hedges et al. 2008). For bees, Koch & Schmid-Hempel (2011b) found that socially-transmitted gut microbiota protect bumble bees against a widespread protozoan parasite, *Crithidia bombi*. And experiments in honey bees have found that lactic acid (*Lactobacillus*) bacteria may protect against infections by *Paenibacillus larvae* and *Melissococcus plutonis* (Forsgren et al. 2010, Vásquez et al. 2012). It is not known if these bacterial groups influence parasitism in solitary bees, which unlike social bees, are not associated with a consistent core microbiota (Martinson et al. 2011, Engel et al. 2012).

We address the hypothesis that the availability of floral resources, nesting materials, and composition of the local bee community influence microbiome variation in solitary bees. Because megachilid bees share bacteria with flowers (McFrederick et al. 2017), forage daily for food and nesting materials, and can be artificially incubated to emerge from pupal casings, they can be experimentally manipulated. We therefore used *Osmia lignaria* as a study organism to test 1) which local and landscape environmental features influence the richness and composition of the bee microbiome, 2) which local and landscape environmental features influence the abundance of bacterial groups associated with immunity in bees (Betaproteobacteria, Gammaproteobacteria, *Lactobacillus* spp., and *Wolbachia* spp.), and 3) if these bacterial groups are associated with reduced parasite prevalence in our study system.

Methods

Characterization of Study Sites

We examined local and landscape characteristics of 17 urban gardens, ranging in size from 444 m^2 to 15,525 m^2, each separated by 2 km, across three counties (Monterey, Santa Clara, and Santa Cruz) in the California central coast. In two sampling periods (early March and early April 2016) we measured local habitat characteristics within a 20 x 20 m plot placed at the center of each garden. We counted and identified all trees and shrubs within the 20 x 20 plot. Then, in each plot we randomly selected four 1 x 1 m plots within which we counted all flowers (from

crops, weeds, and ornamentals), and assessed percent ground cover from bare soil, grass, herbaceous plants, leaf litter, rocks, and mulch. We also estimated the total garden size. For analysis, values were averaged across the two sampling periods. In all, we measured 10 variables: % rock cover, % mulch cover, % leaf litter, % bare soil, % herbaceous plant cover, richness of flowers, abundance of flowers, richness of trees and shrubs, abundance of trees and shrubs, and garden size.

At the landscape scale, we classified land cover types within 500 m buffers surrounding each garden with data from the 2011 National Land Cover Database (NLCD, 30 m resolution) (Homer et al. 2011). We selected 500 m buffers because while *Osmia lignaria* females have a maximum foraging distance of up to 1,200 m (Guédot et al. 2009), they tend to collect more pollen and nectar at flowers near to their nests within 500 m. (Williams & Tepedino 2003). We created four land-use categories: 1) natural habitat (composed of deciduous [NLCD number 41], evergreen [42], and mixed forests [43], dwarf scrub [51], shrub/scrub [52], and grassland/herbaceous [71]), 2) open habitat (composed of lawn grass, parks, and golf courses [21]), 3) urban area (composed of low [22], medium [23], and high intensity developed land [24]), and 4) agriculture area (pasture/hay [81] and cultivated crop [82]). Other land cover types that covered <5% of the total area at each site were not included. We assessed land cover with spatial statistics tools in ArcGIS v. 10.1.

Bee Community Diversity Assessment

We used bee richness data collected for two prior experiments conducted by our research team at these sites (Quistberg et al. 2016, Plascencia & Philpott 2017). Bee community diversity at each site was measured across six sampling periods between June and September 2013 and between June and September 2015. We used aerial nets for 30 minutes (not including handling time) each at site and three pan traps for 8 hours, and netted and placed traps within the 20 x 20 m vegetation plots. We identified bees using dichotomous keys to genus, and when possible, to species (see Quistberg et al. 2016, Plascencia & Philpott 2017 for details on bee sampling and identification methods). For analysis, values were averaged across sampling periods and then across years for each site. In June 2016, we conducted one visual survey for bees for 30 min at each site to confirm that the relative ranking of species richness and abundance was similar across years.

Bee Installation

Over the course of three days in mid-March 2016, we installed *Osmia lignaria* at each community garden. We placed one UV-sterilized binderblock laminate nest (Pollinator Paradise, Parma, ID) at or near the center of each site and each binderblock was stocked with 100 females and 150 males. We applied three sprays of mason bee attractant on each nest (Crown Bees, Woodinville, WA). Bees were allowed to emerge and forage for 16 days. When then collected adult female bees. Each bee was placed into a sterile 2 ml vial and immediately stored in dry ice. We also collected a blank, no-template control air sample at each site. We sterilized

gloves, forceps, nets between collecting each bee and between each sampling site with bleach then ethanol. Bees were transported to the lab and into -80 °C cold storage.

Control Treatment

To confirm that the environment confers unique bacterial communities to foraging bees, we allowed six female bees to emerge from their pupal cocoon casing in a sterile, indoor lab environment in petri dishes. Upon emergence, each female was immediately collected in a sterile 2 ml vial and placed into -80 °C cold storage. We also removed and collected an additional six females from their pupal casing by cutting individual pupal casings with a blade and removing the female with forceps. We also collected their pupal casings for analysis. We sterilized gloves and forceps between samples.

Illumina 16s Sequencing

We collected a total of 344 *O. lignaria* (an average of 19 bees per site). We extracted DNA from each sample and 1 control blank per site with the Qiagen DNeasy blood and tissue extraction kit (Qiagen, Valencia, CA), but with the addition of tissue lysing step using sterile 5mm stainless steel beads and 0.1 mm glass beads in a Qiagen Tissue Lyser II to ensure extraction of gram positive bacteria (Engel et al. 2013). We used whole-insect samples without surface sterilization (Hammer et a. 2015). Library preparation and sequencing (Illumina MiSeq 2X300 with V3 reagents)

was performed using previously published protocols (McFrederick and Rehan 2016). To amplify the 16s rRNA gene, we used the 799F (5′-GAGT TTGATCNTGGCTCAG-3′) and 1115R (5′-GTNTTACNGCGGCKGCTG-3′) primer pair and included negative controls (control blank samples).

Parasite Detection

We screened all *O. lignaria* bees for the presence of fungal *Aspergillus* spp. (Stonebrood) the neogregarine protozoan *Apicystis* spp., and the trypanosomatid protozoean *Crithidia* spp. We used parasite specific primers and conditions for genus-level identification (Table 3.1). Products were run alongside a standard ladder on a 1% agarose gel stained with GelRed to confirm amplicon size. Each assay included a negative and positive control.

Data Analysis

We used QIIME to demultiplex and filter sequence reads (Caporaso et al. 2010). We first used USEARCH to check and remove chimeras (Edgar 2010), then applied SUMACLUST to cluster operational taxonomic units (OTUs) at 97% sequence identity (Kopylova et al. 2016). We assigned taxonomic identity to sequences using the RDP naïve Bayesian classifier (Wang et al. 2007). We removed rare OTUs present at fewer than four reads per sequencing run and removed mitochondrial and chloroplast sequences. We removed any bacteria that are commonly present as sample contaminants and also found in our blanks, such as

Propionibacterium (Salter et al. 2014). To confirm taxonomic assignments, we used BLAST to compare representative bacterial sequences against the NCBI 16s database (December 2015). We confirmed the identity of the 30 most abundant and 30 most frequent OTUs. We used PyNAST (Caporaso et al. 2009) to align candidate sequences to the best-matching sequence in a pre-aligned database of template sequences (Greengenes). We used Mesquite to visually filter alignments to remove highly variable regions and gaps (Maddison & Maddison 2015). After filtering, we calculated alpha (within sample) and beta (between sample) diversity in QIIME and used R to visualize results (R Core 2018). To account for variable sequencing depth, we subsampled to 5,320 reads per sample. This allowed us to retain most samples and capture the majority of sequence diversity found in our samples.

To determine which environmental site characteristics to include in analyses, we selected two variables reflective of floral resources at a site (abundance of flowers and the abundance of trees and shrubs), one variable describing nesting materials (% bare soil), one variable describing the landscape cover (% natural cover within 500 m), and one variable describing the bee community (bee richness). We natural log transformed variables that did not meet conditions of normality. To test for multicollinearity, we calculated a variance inflation factor (VIF) using the car package (Fox et al. 2017) and found each predictor had a VIF score below 2.

To compare the microbiome communities of bees allowed to forage and bees from our control treatment, we performed non-metric multidimensional scaling weighted by abundance and calculated the statistical significance of treatment groups

using the distance matrix with the Adonis method in Qiime. To analyze how floral abundance, tree and shrub abundance, natural cover within 500 m, bare soil, and bee richness influence the variance between microbiome communities of experimental foraging bees at different sites, we first used Qiime to calculate weighted UniFrac distances (Hamady et al. 2010), then performed non-metric multidimensional scaling weighted by abundance using metaMDS in the ecodist package in R (Goslee & Urban 2017). We analyzed the variance between bee microbiomes using the vegan package in R (Oksanen et al. 2015): we used Adonis on the dissimilarity distance matrix and ENVFIT to fit environmental vectors onto the ordination. We then calculated dissimilarity between vegetation communities at each site using the Bray-Curtis method with the vegdist function in the vegan package. We compared the dissimilarity between vegetation communities and the bee microbiome communities at each site using a Mantel test, with 999 permutations. We obtained a Mantel statistic describing the correlation between matrices based on the Pearson method and plotted the correlogram with the mgram function in ecodist.

To analyze how site factors influence microbe diversity and parasite prevalence, we used Qiime to calculate the overall richness of operational taxonomic units (OTUs) given our subsampling depth. We averaged ten estimated iterations of richness for each bee. We also calculated rarified abundance counts of bacterial sequences in the following taxonomic groups for each bee: Betaproteobacteria, Gammaproteobacteria, *Lactobacillus*, and *Wolbachia* OTUs. We then averaged overall OTU richness and abundances of these four bacterial groups across bees at a

site. We calculated the percentage of individuals with each parasite in each garden and averaged this value to obtain parasite prevalence in our overall sample population. We then used generalized linear models (GLMs) with the glm function in R to examine the relationship between the prevalence of each parasite and the abundance of each bacterial group. We modeled a two-vector response variable (infected individuals, not-infected individuals) using the cbind function to maintain information about sample size and required a binomial error structure.

We used generalized linear models with the glm function in R to examine relationships between bacterial counts and the site variables (floral abundance, tree/shrub abundance, % natural cover within 500 m, % bare soil, and bee richness). We tested combinations of these variables using the glmulti package (Calcagno and de Mazancourt 2010) and a Gaussian error structure, with bacterial abundance of Betaproteobacteria, Gammaproteobacteria, *Lactobacillus*, *Wolbachia*, and overall bacterial OTU richness as our response variables. For models where the AICc for top models was within 2 points of the next best model, we ran model averages with the MuMIn package (Barton 2012). When the best models shared the same significant predictors as model averages, and we reported output from best models. To determine the goodness-of-fit of the best models, we calculated a pseudo-R^2 value as [(null deviance - residual deviance)/ null deviance] (Dobson and Barnett 2008).)

Results

Control Treatments

There was a significant difference between the microbiomes of samples within each treatment group: bees allowed to forage in urban gardens, bees reared in sterile environments, bees dissected from cocoon pupal casings, and cocoon pupal casings. (Fig. 1, $p<0.001$, $R^2 = 0.05$).

Beta Diversity & Environmental Context

Environmental variables related to floral resources, landscape context, and bee diversity correlated with differences in microbiome composition of foraging bees. The variables significantly associated with differences between microbial communities and as represented as vectors in the NMDS ordination were percent natural cover within 500 m ($R^2 = 0.06$, $p<0.001$), the number of trees and shrubs in the garden ($R^2 = 0.02$, $p<0.05$), and bee species richness ($R^2 = 0.03$, $p<0.05$) (Fig. 2). Variance in the dissimilarity matrix was significantly explained by the same variables: percent natural cover within 500 m ($R^2=0.01$, $p<0.05$), the number of trees and shrubs in the garden ($R^2=0.01$, $p<0.05$), and bee species richness ($R^2=0.02$, $p<0.01$). We found a significant relationship between the environmental dissimilarity matrix and the bee microbiome dissimilarity matrix ($r=0.06$, $p<0.01$): the positive but small coefficient r indicates a weak correlation between distance matrices, suggesting that microbial communities from bees from similar environments are more similar from one another than those in bees from dissimilar vegetation groups.

Alpha Diversity & Environmental Context

Prior to rarefaction, we found 42,104 distinct OTUs across the bees from all the sites. The mean OTU count per sample was 3108.94 ± 1199.35 (SE). Approximately 1,000 OTUs were responsible for 95% of the bacterial abundance present. Environmental variables were significantly associated with rarefied OTU counts (Table 3.2). *Wolbachia* abundance was higher in sites with higher bee richness ($t=2.44$, $p<0.05$, Fig.3a) and a higher percentage of natural cover in the landscape (500 m) ($t=2.71$, $p<0.05$, Fig. 3b). Betaproteobacteria abundance was higher in sites with high bee richness ($t=2.29$, $p<0.05$, Fig. 3c) but Gammaproteobacteria abundance was not significantly predicted by any environmental variable. *Lactobacillus* abundance was higher in sites with higher floral abundance ($t=2.38$, $p<0.05$) (Fig. 3d). OTU richness was higher with higher bare soil at a site ($t=2.78$, $p<0.05$) (Fig. 3e).

Alpha Diversity & Parasite Prevalence

Crithidia, *Apicystis*, and *Aspergillus* were present at varying rates in bees across the sites (Table 3.3). The prevalence of *Crithidia* was lower in sites where more bees had Betaproteobacteria ($z=-2.69$, $p<0.01$, Fig. 3.4a) but higher in sites where more bees had Gammaproteobacteria ($z=2.54$, $p<0.05$, Fig. 3.4b). The prevalence of *Aspergillus* was higher in sites where more bees had *Wolbachia* ($z=2.41$, $p<0.05$, Fig. 3.4c). The prevalence of *Apicystis* was lower in sites where

more bees had *Lactobacillus* (z=-1.94, p=0.053, Fig. 3.4d), although this relationship was marginally significant.

Discussion

Environmental variables related to resource availability, landscape context, and bee diversity influenced the composition of the bee microbiome. This is in contrast to previous a previous study that found that agricultural land-use has little to no impact on the microbial communities in social bumble bees (Cariveau et al. 2014). Our study design differs because we measured fine-scale, local variables, such as floral abundance and groundcover characteristics, whereas Cariveau et al. (2014) addressed the impact of categorical habitat types on microbiome composition. It may be easier to detect and compare the impact of environmental variables on the microbiome of this species of megachilid bees because they lack the core bacteria commonly found in social bees, because they have shorter foraging ranges than social bees, and because they can be manipulated to emerge and forage within the same time frame across multiple sites. Environmental features such as diet and geography are more influential than host genetics for microbiome variation in humans, mammals, and flies (Goodrich et al. 2016), but for bees, this is the first study to even confirm that that environmental variation influences both the composition of the microbiome and the relative abundance of particular bacterial groups.

During metamorphosis, bees undergo gut reorganization in which the larval gut is shed (Hakim et al. 2010). However, we found that bees reared in sterile

environments are not “blank slates”, but are host to a microbiome that is significantly different in composition to the microbiome of foraging bees (with the caveat that we examined relative abundance, not absolute abundance). Therefore, while some bacteria are likely acquired at some point before or during bee emergence from their pupal cocoon, we find that the adult bee microbiome is shaped by interactions with the environment.

We found that bee richness at a site was associated with abundance of the socially-transmitted Betaproteobacteria, which in turn was associated with lower prevalence of *Crithidia*, a widespread parasite of multiple bee species. This finding supports Koch & Schmid-Hempel’s (2011b) argument that the microbiome can be considered an “extended immune phenotype”, and suggests that horizontal transmission of bacterial symbioses may be important for bee health. While some bacteria within the clade Betaproteobacteria are transmitted through contact and fecal exposure, it is unclear how bee richness facilitates the association between bacteria and the bees. An increase in bee species may increase the likelihood of within and between species interactions during foraging trips for food and nesting materials, facilitating bee-bee transmission of bacteria. It may also increase the likelihood that a foraging bee is exposed to materials previously touched by other bees or exposed to bee feces left in the environment. Our finding that Betaproteobacteria was associated with both bee richness as well as lower *Crithidia* infection suggests that not only social bees, but solitary bees too may benefit from social interactions. However, these findings are complicated because we found that neither bee richness nor any

environmental features predict the abundance of the socially-transmitted Gammaproteobacteria, which was associated with higher *Crithidia* infection. More work is therefore needed to determine which specific OTU types within the Betaproteobacteria and Gammaproteobacteria influence infection outcomes.

In non-bee insects, *Wolbachia* has been associated with impacts to host reproduction as well as fitness benefits to hosts infected by RNA viruses (Hedges et al. 2008). Because Wolbachia is a widespread intracellular bacterial associate of insects but has not been associated with changes to reproduction in social Hymenopterans (Wenseleers & Billen 2000), we expected that *Wolbachia* association might positively influence bee-parasite interactions. However, we found that *Wolbachia* abundance was associated with a higher prevalence of the fungus *Apsergillus* in bees. It is possible that increased parasite prevalence acts synergistically with other, unknown *Wolbachia* associated traits in bees; more research is needed on the role of *Wolbachia* in bee health.

Bare soil groundcover in the gardens was associated with higher overall OTU richness in the *O. lignaria* microbiome. Given that *O. lignaria* bees collect and transport wet soil between their mouthparts multiple times a day to construct cell chambers in their nest, a higher abundance of bare soil patches or area could mean that bees are collecting soil from a larger spatial area of soil, thereby increasing contact with a greater diversity of microbes. In a previous study in this same study system, bare soil in urban gardens was also associated with higher bee species richness (Quistberg et al. 2016), possibly because multiple species nest below-ground

or use soil as a nesting material. An abundance of bare soil may indirectly impact OTU richness if it attracts and concentrates bees into an area wherein they are then likely to exchange microbes. Although OTU richness was not associated with parasite prevalence, the role of nesting materials for bee diversity and bee-microbe interactions is often overlooked; our findings call for further research on the ecological role of nesting resources in shaping the microbiome.

We contribute to the growing body of literature highlighting the dual role of flowers in mediating bee health. Flower diversity can benefit bees. For example, bees fed poly-floral diets exhibit a higher expression of immune-related genes (Mao et al. 2013) and lower mortality when challenged by some parasites (Di Pasquale et al. 2013). But flowers can also host parasites and pathogens: both RNA viruses (Singh et al. 2010) and parasitic *Crithidia* spp. (Graystock et al. 2015) can be florally transmitted within and between bee species. Finally, flowers act as bacterial transmission hubs, with wild bees acquiring bacteria from floral interactions (McFrederick et al. 2012, McFrederick et al. 2017). We found that higher floral abundance in urban gardens is correlated with increased *Lactobacillus* abundance in *Osmia lignaria,* confirming that the environment plays a role in shaping *Lactobacillus*-bee interactions. One question remaining is how lactic acid bacteria influences bee fitness. Because some *Lactobacillus* species protect bees against fungal parasites in laboratory settings (Forsgren et al. 2010, Vásquez et al. 2012), we expected to find an association between lactic acid bacteria and infection by the fungus *Aspergillus*. However, we only found a marginally significant beneficial

association between *Lactobacillus* and *Apicystis* (p=0.053). We suggest more research on the possible protective benefits of the *Lactobacillus*, as it has previously been proposed as medicinal probiotic for bees (Evans & Lopez 2004). Finally, while bee-friendly initiatives recommend growing flowers for bees, no study has yet addressed the fitness tradeoffs between immunity benefits, microbial associations, and exposure to flower-associated parasites and pathogens for bee health. Future experiments examining which flower species and which flower traits are important for parasite, pathogen, and microbial associations may reveal some of these tradeoffs and inform decisions around which flowers to plant for bees.

While other studies have confirmed the presence of *Crithidia* and *Aspergillus* (Stonebrood) in megachilid bees, this is the first report of the neogregarine *Apicystis* in a species from the genus *Osmia*. The impact of these three parasites in megachilids is largely unknown, and our findings only confirm that they are present. Because *Osmia lignaria* is increasingly adopted for commercial pollination, it is important to know if these parasites actively replicate, infect, and harm megachilid bees. Finally, although we examined if microbial composition influences parasite prevalence, we did not assess how bee fitness is directly impacted by environmental context. For example, local and landscape features in an agricultural site can impact bee health directly through variability in food quality and quantity, or indirectly through impacts to bee physiology. Although local and landscape features in agricultural landscapes are associated with fitness-related measured such as bee size, bee fat content, and nesting density (Wood et al. 2017), the role of the microbiome remains elusive. We

suggest more research on how the microbiome impacts the nutritional state of bees (Borer et al. 2013, Gibson & Hunter 2010). This work may reveal changes in the microbiome associated with the landscape could have indirect impacts to bee-parasite interactions.

Authors' contributions

HC acquired funding for the research, led study design, fieldwork, lab work, and coordinated manuscript writing and publication. QRM contributed to field research design, provided physical and financial access to laboratory equipment, supervised laboratory methods, and contributed to the manuscript. SMP contributed to field research design, fieldwork logistics, data analysis, and manuscript writing.

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Table 3.1 PCR mixes and conditions for the detection of parasites.

Primers	Primer Name	dNTP (µl)	Taq (µl)	10x Buffer (µl)	Primer F (µl)	Primer R (µl)	Template (µl)	Total vol. (µl)	1 Denaturing Min | Temp	2 Replication Sec | Temp	3 Elongation Min | Temp	Amplicon size (bp)
Apicystis spp. (Meeus et al. 2010)	NeoF, NeoR	0.2	0.05	1	0.2	0.2	1	10	2 | 95	35x 30 | 94 30 | 60 45 | 72	3 | 72	850
Crithidia spp. (Meeus et al. 2010)	SEF, SER	0.2	0.05	1	0.2	0.2	1	10	2.5 | 95	35x 30 | 94 30 | 56.5 52 | 72	4 | 72	417
Aspergillus spp. (Williamson et al. 2000)	AF4, AR1	0.2	0.05	1	0.2	0.2	1	10	3 | 94	30x 30 | 94 40 | 58.5 50 | 72	7 | 72	222

Table 3.2 Results of GLM model selection for bacterial groups. Table shows significant variables from the best model selected[§].

Dependent Variable	Predictor(s)	AIC	pseudo-R^2	df
Betaproteobacteria	Bee richness*	216.78	0.260	16
Gammaproteobacteria	none			
Lactobacillus OTUs	Floral abundance*	160.96	0.413	16
*Wolbachi*a OTUs	Natural cover 500 m (%)*, Bee richness *	163.69	0.471	16
OTU Richness	Bare soil (%)*	211.78	0.557	16

[§]All relationships featured in the table are positive, there were no negative predictors. (Signif. code:'*' 0.05).

Table 3.3 Infection prevalence rates for each parasite.

	No. infected	n	Percent (± SE) infected
Crithidia spp.	98	344	45.01 ± 1.33
Apicystis spp.	155	344	28.49 ± 0.87
Aspergillus spp.	272	344	79.07 ± 0.94

Figure 3.1 NMDS Plot of Weighted Unifrac Abundances. Samples represent the microbial community of bees from experimental, foraging treatments in urban gardens and bees and pupal casings from control treatments

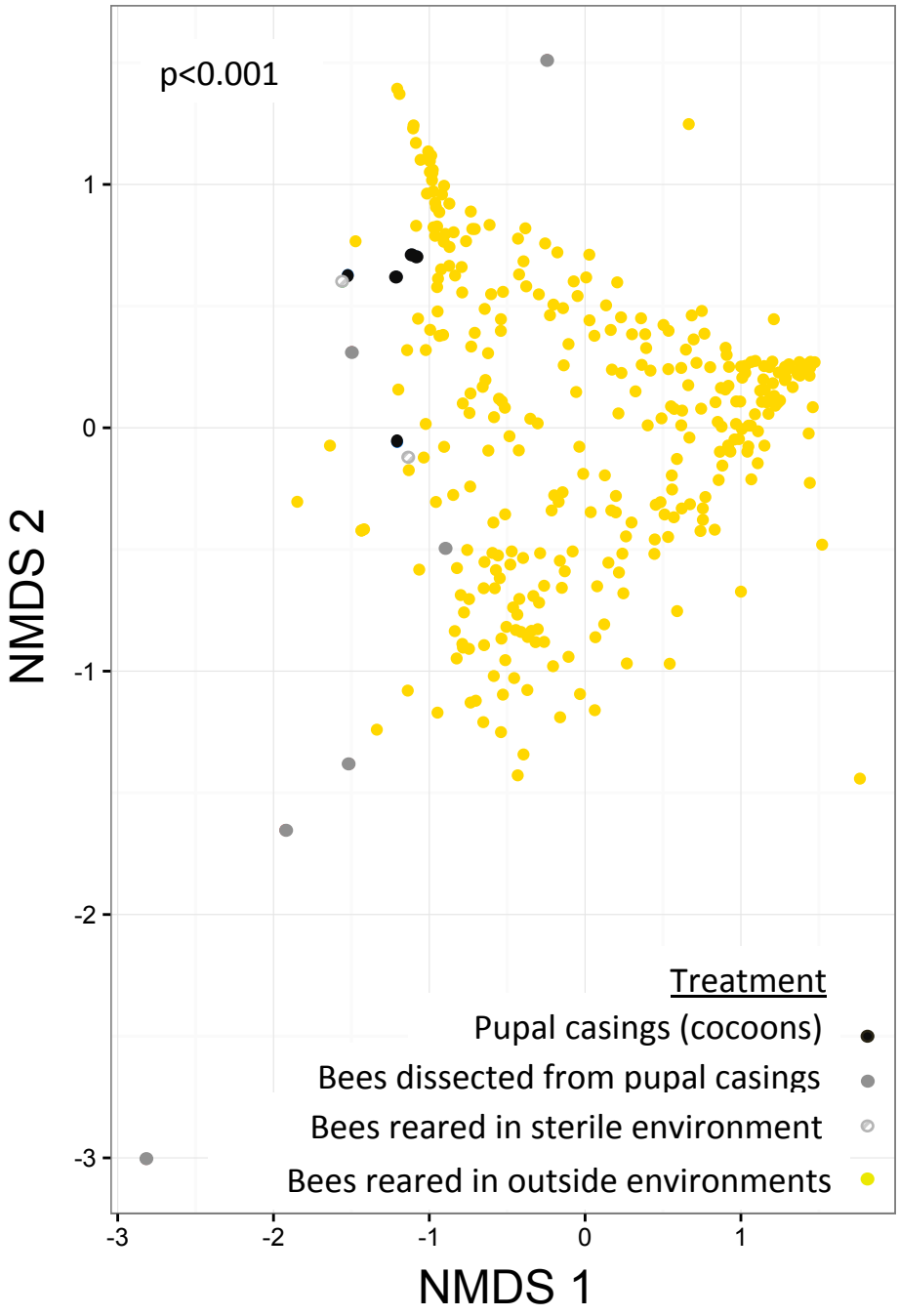


Figure 3.2 NMDS Plot of Weighted Unifrac Abundances. Each sample represents the microbial community of bees from experimental, foraging treatments in urban gardens. Each vector reflects the variables associated with differences between microbial communities. Significance values determined by ENVFIT function. (Signif. codes: '***' 0.001, '**' 0.01, '*' 0.05, ' ' 1).

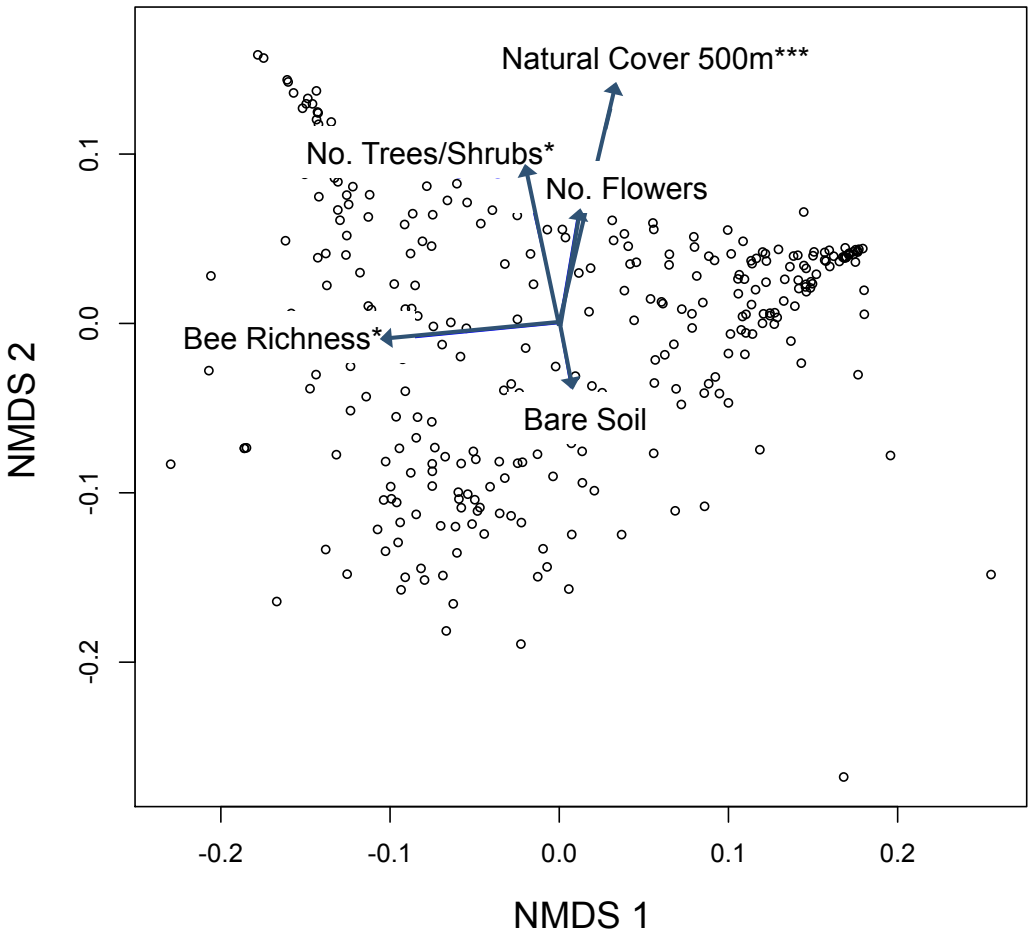


Figure 3.3 Results of GLM model selection for bacterial groups. Graphs show significant variables from the best model predicting the average abundance of *Wolbachia* spp. (a,b), Betaproteobacteria (c), *Lactobacillus* spp., (d), and the richness of distinct OTUs (e) in each bee.

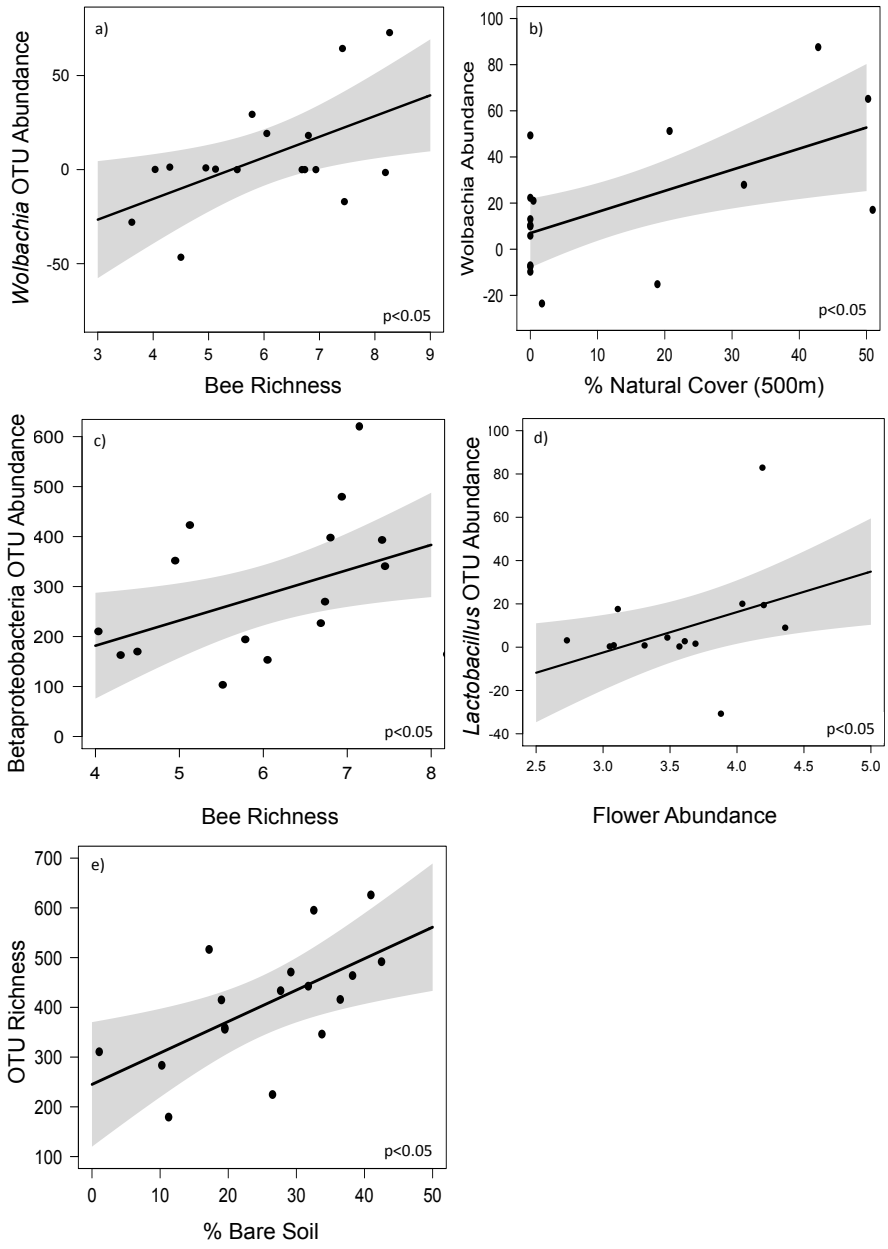
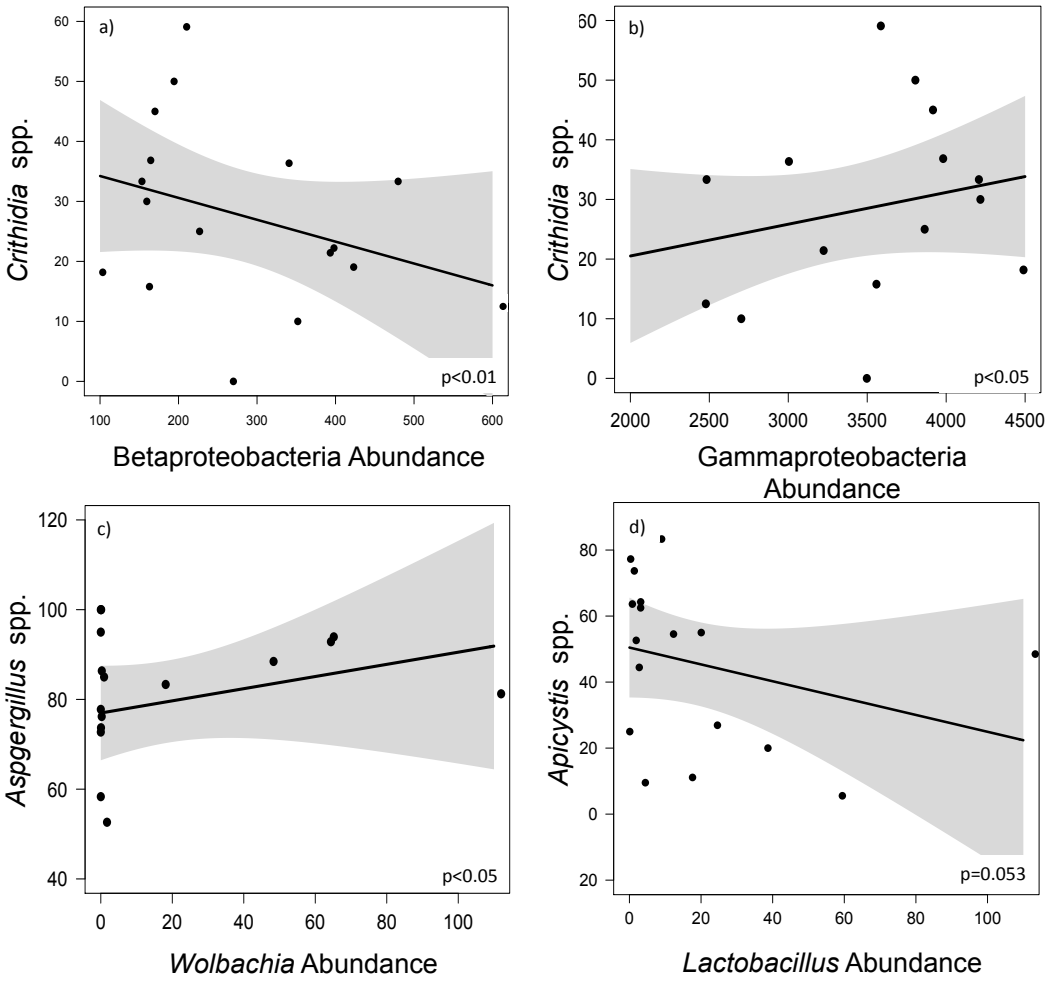


Figure 3.4 The prevalence rates of *Crithidia* spp. (a,b), *Aspergillus* spp. (c), and *Apicystis* spp. (d) are influenced by the abundance of Betaproteobacteria, Gammaproteobacteria, *Wolbachia*, and *Lactobacillus* OTUs. Each individual graph represents the output of a general linear model glm (y ~ bacteria abundance, family=binomial).



CHAPTER 4: Hiving-off risk in California almonds: Appropriationism and the orchard beekeeping industry

Abstract

This chapter uses Marxist theories of agrarian capitalism to explore the political economy of orchard beekeeping, a nascent industry in the California almond industry developing in response to honey bee population declines. Marketing pollination to the farmer is an example of what agrarian political economists have called appropriationism, the transformation of aspects of agricultural production into discrete industrial inputs. The article argues that theorizations of the structural requirements of capitalism in agriculture fail to account for the unique presentation of appropriationism in the orchard beekeeping industry: while appropriationism putatively leaves risky-aspects of farm production to the farmer, orchard beekeeping remains an incredibly risky business. This article enumerates the risks and challenges of orchard beekeeping, contending that it is different from other appropriationist innovations.

Introduction

The central valley of California produces over 75% of the world's almond supply, with over 6,000 growers maintaining over a million acres in production (CDFA 2016). Almonds need bees for pollination, but bee populations are dying due to a combination of pesticides, habitat loss, and disease (Goulson et al. 2015). Growing California almonds therefore requires the temporary installation of half of

the US honey bee population during bloom periods. Honey bees are shipped to the central valley to pollinate almonds each February. However, honey beekeepers have lost 44% of their migratory colonies in the last year due to Colony Collapse Disorder, and the price of honey bee pollination rentals for almonds has nearly doubled since 2006 (USDA, 2017). With increasing concern over honey bee losses and high prices (almond farmers spend more on beekeeping costs than on irrigation), many almond growers and agricultural researchers have turned to the domestication of native orchard bees for insurance against pollination losses.

Pollination by bees is essential for accumulation and production in the almond industry, but presents a natural challenge to farmers because bees cannot survive on industrialized, monoculture farms. As an alternative to using honey bees, growers purchase orchard bees from specialized orchard beekeepers, outsourcing the process of pollination. Marketing pollination to the farmer is an example of what Goodman et al. (1987) have called appropriationism. Writing in the neo-Marxist tradition of agrarian political economy, Goodman et al. defined appropriationism as the transformation of aspects of agricultural production into discrete industrial inputs that would be purchased by the farmer. While other scholars in agrarian political economy have added that appropriationism leaves the most risky aspects of farm production to the farmer, the research reported here suggests that orchard beekeeping remains a highly risky business.

In this chapter I examine how beekeepers transform pollination into an industrial input and then examine the risks they face in doing so. This chapter thus

locates beekeeping within the historical context of agrarian capitalism, linking with debates about the challenges that agricultural production poses to capitalism. I contend that orchard beekeeping differs from earlier appropriationist innovations because the distribution of risk in almond production is not placed only on the farmer, but on the appropriator, in this case the beekeeper.

Orchard bees emerge as a solution to honey bee losses

While there has been a resurgence of recent interest in the adaptation of orchard bees for fruit and nut production, the diversification of pollination markets is not a new phenomenon: breeding programs in the United States, Canada, and Europe have promoted the adoption of leafcutter and bumble bees as alternatives to honey bee as early as the 1960s. But it is important to examine the industrialization of orchard beekeeping particularly because it is the only alternative to the honey bee available for commercial pollination of almonds in California. The Blue Orchard bee, *Osmia lignaria*, is a metallic blue-black solitary bee used for almonds because it is early-season pollinator that overwinters in a small, hardy pupal cocoon. Orchard beekeepers bring thousands of orchard bee cocoons and nesting blocks or reeds to almond farms just before bloom. After the bees emerge and mate, the females will utilize the nests provided by beekeepers. Females create chambered mud cells within the nest and provision each cell with a pollen ball and egg, laying up to 6 female eggs in their 8 week lifespan (Bosch & Kemp 2001). Their eggs turn into larvae, which eat the pollen, then pupate, spending autumn and winter as adults inside their cocoons.

After bloom, beekeepers return to collect the nests and harvest the cocoons to sell back to the farmer the following year.

Orchard bees, as "alternative" pollinators to the honey bee, have unique features that present both challenges and opportunities for domestication (Boyd et al. 2001). Although blue orchard bees are solitary by nature, they are also gregarious, and therefore willing to nest in close proximity to one another, allowing for management (Bosch & Kemp 2001). They are extremely efficient pollinators, with only 250-300 females required to pollinate an acre (versus 100,000 honey bees). They are supposedly resistant to many of the pathogens responsible for honey bee decline. While honey bees must be transported as temperamental adults, orchard bees are easily shipped across state lines as cocoons that cannot sting, escape, or protest transportation.

The orchard bee industry took off after 2006, initiated in response to honey bee declines. Businesses are located primarily across the western US, in Utah, Idaho, Washington, Oregon, and California. Several beekeepers report multiple facilities across these states. Beekeepers sell to three different kinds of clients. The majority of beekeepers supplement their commercial orchard pollination business by selling or renting *Osmia* bees to retail clients, i.e. backyard gardeners that grow fruit trees in an area of land that is under an acre in size. A few beekeepers also hold wholesale arrangements with distributors such as plant nurseries and garden stores. Finally, commercial beekeepers sell or rent to commercial growers. Regardless of the beekeeper's location, the majority work with growers in the central valley of

California, followed by growers in Washington, Oregon, and Utah. Growers include predominately small and medium-scale commercial almonds, apples, cherries, and other berries and fruits, but clients also include the large-scale almond farms whose fields blanket the central valley of California.

Lineages of the agrarian question, appropriationism, and beekeeping

Marxist theorizations of capitalism have sought to understand how capitalism penetrates agriculture. Marx theorized that material conditions and modes of production develop in successive linear historical stages. Following primitive accumulation comes a slave society, then feudalism, which is superseded by capitalism. Capitalism is a necessary historical epoch before society can transition to the final successive stage, socialism. Karl Kaustky, writing *The Agrarian Question* in 1989, applied this Marxist theory to understand the role of agriculture in the trajectory of capitalist development. Kautsky found that agricultural production was an anomaly to the assumed capitalist development of industry (Banaji 1976). Characterized by small-scale peasant agricultural holdings, agricultural production would stunt the historical trajectory towards socialism. But how could this be? Since Kautsky's "agrarian question" and claim that small farms are at odds with capitalist development, agricultural exceptionalism has become the focus of acute academic debate. In confronting the contradictions between agriculture and Marx's theory of history, several scholars address how conceptions of nature are particularly important

to addressing the agrarian question and the penetration of capitalism into agricultural production.

Susan Mann & James Dickinson revisited the agrarian question in the article *Obstacles to the Development of a Capitalist Agriculture* (1978). Mann and Dickinson observed that the small family farm and family labor have persisted, contradicting trends in other parts of capitalist economy. In her book *Agrarian Capitalism in Theory and Practice* (1989), Mann elaborated on the Mann-Dickinson theory, arguing that the natural characteristics of agriculture make it intractable to industrialization and capitalism. Mann noted that capitalism cannot contend with "capricious" and "erratic" nature: the very material conditions of farming make it difficult for capitalists to realize profits (p. 33). Agriculture depends on long growing seasons, livestock reproductive lifecycles, and sharp peaks and valleys of labor demand (40), and this is an obstacle for capitalism.

It is in the context of these discussions on the agrarian question that Goodman et al., in their book *From Farming to Biotechnology* (1987) developed a framework for understanding how capitalism might overcome spatial, temporal, material, and biophysical barriers in nature. For Goodman et al., industrialization faces three constraints in agriculture: nature as the biological conversion of energy, nature as biological time in plant growth, and nature as space in land-based rural activities. Capitalism works around the farm with two accumulation strategies, appropriationism and substitution. Appropriationism refers to efforts of firms to transform aspects of agricultural production into industrial inputs, whereas substitutionism refers to efforts

of firms to develop industrial substitutes for agricultural end-products (p. 2). An example of appropriationism is the transformation of the natural properties of soil and organic matter into industrially produced fertilizer (p. 29), which is then sold back to the farm. Through these strategies, capitalism penetrates agriculture not through the cultivation process, but in upstream and downstream industries that fuel production. Appropriationism and substitutionism have had three results according to Goodman et al. First, they serve to transition farming from extensive in form to intensive concentration (p. 56), facilitating the production of higher yields per area rather than taking over new land. Second, farmers become price-takers. If industry provides inputs, it has the power to set prices that farmers have to accept. This places farmers at whim of buyers and suppliers, as well as in competition with other farmers. Finally, processes that industries classify as risky are left on the farm, while lower-risk agricultural activities take place off the farm (Little and Watts 1994, Boyd and Watts 1997, Buck et al. 1997).

At first glance, orchard beekeeping instantiates appropriationism because it involves removing a production process off the farm and then selling it back to farmers as a discrete input. As such, one might assume that beekeeping is a low-risk activity. Yet, one only need look at the example of Wonderful Farms to suggest that this is not the case.

Wonderful Farms is the world's largest almond grower. In 2009, Wonderful Company established a private, experimental orchard bee program in collaboration with USDA-funded researchers at UC Davis. They invested heavily in R&D, staff,

land, and facilities to optimize nest design, manipulate bee phenology to match almond bloom, and quantify the pollination provided by orchard bees in comparison to honey bees. However, at the time of writing this manuscript, the orchard bee program at Wonderful Company was in the process of shutting down without public explanation. My research provides some clues about why this event might have taken place.

Methods

As part of a broader project on the impact of agricultural practices on disease dynamics in *Osmia lignaria*, I acted as a participant-observer at two annual meetings of the Orchard Bee Association (OBA) between 2016-2017. The OBA is a non-profit organization promoting the orchard bee for commercial pollination of almonds, apples, and stone fruits. In the annual meeting, commercial beekeepers, hobbyists, and land-grant scientists promoting orchard bees meet to discuss the future of the industry. At each meeting I presented the results of my research on disease dynamics in orchard bee systems and served on the outreach committee. I conducted 13 interviews with beekeepers to determine risk and challenges associated with the industry. The interviewees were selected based on interactions at the Orchard Bee Association, willingness to participate, and references from other beekeepers during interviews. Each interview was conducted over Skype and recorded with the interviewee's verbal consent.

I used a semi-structured interview format (Longhurt 2003), beginning with general questions about their business structure, clientele, and background. To understand general management practices comprising the industry, I asked each interviewee to describe their day-to-day and how their understandings of best practices have changed over time. I asked each beekeeper to describe the risks and challenges they face. At the end of each interview, I invited the interviewee to tell me anything I may have missed. Each interview lasted for 60-90 minutes. Interviews were transcribed and coded using NVIVO software. Before coding, I conducted pre-coding by highlighting significant quotes and querying transcripts for the most commonly expressed words and phrases (Miles et al. 1994). I coded for phrases describing risk and challenges. I also coded for phrases indicating collaboration, competition, or conflict with other actors. To describe management practices in response to risks and challenges, I coded the transcripts for phrases describing practices along the bee life cycle, such as phrases describing decision-making processes and lessons-learned. Although it was not my original question to address how beekeeping as an appropriationist solution differs from other innovations and transformations of production, I found that beekeeping challenges theories of appropriationism because of the many risks and challenges enumerated by beekeepers.

Beekeeping is risky

> The problem with growing bees in orchards is that you can't grow bees in orchards.

The theory of appropriationism suggests that when beekeepers sell pollination to the farmer, beekeepers retain the most predictable and profitable aspects of the pollination system while farmers are left with the risk. My discussions with beekeepers highlight that beekeeping is hardly a risk free business. In what follows I enumerate four risks associated with natural obstacles: weather, low nest establishment, fragmented wild populations, and vulnerability to pesticides from surrounding farms. I then highlight a risk associated with the social relations of capitalism, an additional anomaly to the theory of appropriationism: tensions with farmers.

Weather

> Weather's number 1. By far. And we can't control that. Weather has to do more with returns than any other factor.

One of the main risks to agrarian production, across the board, is weather (Goodman and Watts 2013). It is a risk for small and medium-scale organic producers reliant on good weather to sell at the farmer's market (Buck et al. 1997), for commercial growers in the US and Canada (e.g. Smithers and Blay-Palmer 2001), for farmers in Global South (e.g. Tucker et al. 2010), and it is also a risk for orchard beekeepers. Weather greatly influences nest establishment and cocoon returns and was the most commonly reported challenge across all interviewees. In the wild, orchard bees emerge upon experiencing warm temperature cues in early Spring, but

almonds bloom in February. Beekeepers therefore keep cocoons in cold storage and then artificially incubate bees to initiate emergence. However, due to changing weather, bloom time differs slightly every year. Beekeepers therefore have to predict when to start incubation. If bees emerge too early they may starve due to lack of flowers, if they emerge too late they may miss a critical pollination window. Sudden temperature shifts or rain events after cocoon release can directly kill bees or result in reduced flying hours, compromising both nest establishment and pollination of the orchard. Temperature and humidity are also extremely important for storing bees – incorrectly stored bees or bees that warm during transportation may inadvertently use fat stores, reducing bee fitness (Bosh et al. 2010). Finally, beekeepers report that temperature is important for parasite and pathogen infection – humidity promotes fungal infections and pollen mites, which reproduce in larval chambers and destroy pollen.

Low Establishment Rates

Bees die in almonds. Monoculture almond farms are extreme environments for bees: they are exposed to pesticides, dust, tilling, and other disturbances. Although bees live for up to 8 weeks in the wild, they die prematurely from starvation on almond farms which can only provide bloom for 3 weeks. On the farm, bees often opt not to establish in provided nests, or they do but die during bloom due to agrochemical exposure, errors arising from miscommunication with growers, or weather events. Beekeepers thus report that a 30-50% establishment rate of released

bees is considered good return. Low nest establishment rates in monoculture orchards is a key risk for beekeepers because they simply cannot propagate enough bees to ensure enough cocoons to sell back to the same farmers the following year or to grow their business. Explains one keeper, "if you try to increase them in an almond orchard, it's probably not going to work well. It's just not. Even an organic orchard." The vulnerability of bees to orchard management is thus a limiting factor preventing capital accumulation.

Depletion of Fragmented Wild Populations

A third risk that beekeepers face is small, fragmented, and quickly depleting wild populations of orchard bees. Beekeepers compete for a common pool resource: wild bee populations that are captured and brought into domestication. Because bees die in orchards, beekeepers must find another way to supplement their stocks of cocoons to resell to farmers every year. Beekeepers therefore trap from wild populations to offset bee losses on almond farms. Most beekeepers trap wild bees, either directly or through contract with specialized trappers. Trappers place nesting boxes called "trap nests" in a wild area with unmanaged orchard bees. Trapping success can be increased by placing multiple boxes throughout many environments and locations. While the OBA officially recommends that all members gain permission from landowners, whether private or government, each year BLM and Forest Service rangers in Utah contact OBA leadership after finding unpermitted nests strewn along the borders of public land.

> There's always going to be people out there that are cowboys that are just in it for themselves. They're going to be out there sneaking around and extracting bees and not actually managing populations.

Many beekeepers report concerns with illegal trapping because it jeopardizes their own ability to propagate bees. Trapping depletes the wild orchard bee populations upon which all beekeepers depend, including those beekeepers who follow the playbook and trap only on private lands. According to one beekeeper, *Osmia lignara* populations in the western US are naturally small and geographically isolated -- trapped populations are therefore depleted quickly. This beekeeper reported that several locations known to him in northern Utah that were heavily trapped between 1994-2004 have never recovered. No one knows the extent of illegal trapping because most of the wild bees caught are not advertised for sale but pre-sold on a private market. Not only does illegal trapping risky for beekeepers because it depletes wild populations, but consistent trapping of the same populations may have environmental implications: it can alter population genetics, mediate disease transmission between bees artificially concentrated at trap nests, and result in discarded bycatch when non-*Osmia* species establish within the trap nests. These environmental consequences are risky for beekeepers who depend on wild bee as a raw material for commercial pollination.

The Neighbor Problem & Pesticides

> I've seen bees that were working and the neighboring grape farm sprays and the spray floats over the almond orchard and the bees die. I've never had bees that just leave, there's always something that causes them to leave.

> I've seen aerial spray on crops like these artichokes. We had blow over from their pesticides that just whacked like half of our bees. And there were literally just bees dead on flowers. Just frozen, just totally dead. Another time I had my neighbor spray his pesticides right next to my bee nests. And my bees all huddled in, they just starved, they knew there was something going on, and they just stayed in there. And I was like- my bee condos are probably about 10 feet away from the fence and he had mists just going everywhere as he's spraying this pesticide. It just made me furious.

Beekeepers also face risk because they are subject to sprays from neighboring farms. Almond growers will often pause pesticide spray regimes during bloom periods to prevent bee losses during critical pollination windows. Beekeepers report that they work directly with almond growers and farm employees to create spray plans that reduce or limit the application of high toxicity herbicides, fungicides, and insecticides. For example, upon beekeeper recommendations, many farmers will spray at night when bees sleep in their nests, or they will place physical barriers around the nests during sprays to protect bees from spray. But despite communication with growers, beekeepers are subject to risk because they have no leverage over the activity of neighboring farmers. Bees are what ecologists call central-place mobile organisms, foraging across large distances before returning to a nest site (Kremen et al. 2007). Orchard bees have a foraging distance of up to 1,200 m (Guédot et al. 2009), meaning that even if beekeepers release bees on a farm that reduces pesticide sprays during bloom, bees may forage on nearby farmland that is managed by another company. Another problem is that spray may drift or blow over from neighboring

farms on windy days, harming orchard bees. Beekeepers and their bees are thus susceptible to the action of their neighbors with whom beekeepers hold no contract.

A Final Anomaly: Tensions with Growers

> If they weren't spraying a pesticide, they were spraying an herbicide. If they weren't spraying an herbicide, they were spraying a fungicide. Or they were doing mechanical plowing or mowing of weeds. Every 3 days – something. [...] There were several times where I had alternative flowers planted. I found that the bees did much better with multiple resources. But there were several times when the weeders would come in and just mow all the flowers down. It has to do with the farm manager and his priorities.
>
> The reality is, farmers don't care about bees. Growers don't care about bees. They care about their fruit and their crop. If they want pollination, they will call someone and they will demand that you come over there and service them and you leave. That's generally speaking what a farmer is like. They don't want to do self-management. I thought that they would want to because, again, I'm thinking from my mind, of course I want to be self-reliant, I want to do my own stuff. But the reality is they don't. They just want to have cherries. They just want to have almonds.

Theorizations of agrarian populism often assume that those marginalized by capitalism will act as stewards of the land. Therefore it is presumably farmers, not beekeepers, who should have stake in the long-term sustainability of orchard bee pollination. I found that the so-called appropriators in this system, the beekeepers, are the one left caring. Beekeepers commonly reported tensions with farmers who jeopardize the safety of the orchard bees. According to beekeepers, growers do not care about bees, they are only concerned with the bottom line – profits. Beekeepers report that farmers spray herbicides even after agreeing not to, that farmers treat bees as a temporary service, and that renting bees to farmers causes "too many problems,

too much headache." While beekeepers recommend reduced or alternative spray strategies, they are often unable to dictate and control what actually happens in the field. These difficulties, especially around sprays, often result in tensions, conflicts, and reports of anger from beekeepers.

> I think when the farmer owns the bees he takes better care of them. When he rents them, it's just human nature. It's not his fault. It's just human nature, you don't care about them as much and so when you're dealing with sprays and that kind of stuff, so you're more careless. You're just not invested in it. When they own the bees, they really think about what they're doing. So that's why, we, on a commercial level, no longer rent bees. What we're trying to get them to do is change the grower's approach, though. By allowing them to own the bees, that changes their habits.

Grower behavior is not only another challenge or risk that beekeepers face, but has implications for both integration of capitalism into almond production and for understanding notions of stewardship in agrarian political economy. In response to conflicts with growers, beekeepers have reorganized their business structure to both elicit farmer responsibility and redistribute the risk of bee losses between themselves and the farmer. Whereas honey beekeepers historically have rented bees to farmers, orchard beekeepers have shifted towards an ownership model. These beekeepers report that farmers who own bees "have a stake in treating bees well," "good nest establishment, and "take ownership of the bees." One beekeeper described a farmer who developed a novel pesticide application strategy utilizing pesticide helicopters that he reports successfully reduced bee exposure to sprays by 1/3. That beekeepers promote stewardship and actively educe responsibility in farmers is important in the

context of worldwide bee declines. This finding presents an additional anomaly to theorizations of appropriationism, which falsely assumes that appropriators always and only act in accordance with the logic of capitalism.

Conclusion

Beekeepers engage in appropriationism by selling pollination to farmers. But instead of hiving off profit and leaving the farmer to deal with risks, beekeepers themselves report experiencing the risks associated with orchard bee management (weather, the foraging behavior of bees, and the propensity of the bee to die when exposed to pesticides). While theories of agrarian political economy seem to suggest that appropriators act only in accordance with the logic of the market and that that risk-laden farmers act in accordance with the logic of stewardship, I found that the opposite is true. In this system, beekeepers clash with growers over management of the bee. They actively utilize strategies to promote responsible management from growers. I argue that beekeeping is therefore different from other appropriationist inputs. While appropriationism theorizes that bees are transformed into an industrial pollination input to overcome natural obstacles, I found that the challenges, obstacles, and opportunities associated with the innate biology of the bee leave the beekeeper (not the farmer) with risk. This chapter therefore questions the presumed structural contours of capitalism in agricultural production and also brings to light the risks associated when diverse actors stand to benefit or lose from appropriationism.

As a nascent industry, it is yet to be seen if orchard bees will be adopted for large-scale pollination in addition to or in lieu of the honey bee. While beekeepers report strategies to reduce conflict with growers, promote stewardship, and mitigate against bee losses, one question for future study is what are the other and varied ways that beekeepers respond to natural obstacles (such as weather), and what are the implications for bee conservation and the future of the industry? If orchard beekeeping does not make the production process less risky, if it does not evince appropriationism, will it persist?

SUPPLEMENTAL FILES

Two permission files were submitted with this dissertation:

Permissions File 1. Permission from co-author Robyn Quistberg to include a re-print of previously published material in Chapter 1.

Permissions File 2. Permission from Quinn McFrederick and Kaleigh Russell to include co-authored text that is not yet published in Chapters 2 and Chapter 3 of the dissertation.

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