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Ecological Factors Shaping *Nosema* and *Crithidia* Prevalence in Coastal Sage Scrub Bee and Ant Communities

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by

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ABSTRACT OF THE THESIS

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by

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Pathogens are one of the major factors attributed to bee species declines. Flowers can serve as hubs for bee pathogens and shared floral resources mediate the acquisition of pathogens from infected to healthy hosts. While previous research places emphasis on pathogen spillover from managed bees to wild bee species, there is a paucity of knowledge as to the role of non-bee floral visitors, such as ants, in shaping pathogen prevalence for bees. Specifically, it has been previously documented that ant and bee species share viruses. However, whether non-viral pathogens are shared among ants and bees has yet to be elucidated. Here, we document the prevalence of two common non-viral bee pathogens, *Nosema* spp. and *Crithidia* spp., among honey bees, native bees, and ants at an ecological reserve in Southern California encompassing threatened coastal sage scrub habitat to understand how ecological factors shape host-parasite interactions over space and time for these insect taxa. *Nosema* and *Crithidia* were detected in honey bees, native bees, and ants. Surprisingly, both pathogens were highly prevalent ants. Plant-host associations did not appear to be important in shaping pathogen prevalence for honey

bees and native bees. This is the first study, to our knowledge, to detect *Nosema* and *Crithidia* in ants and several native bee species. We discuss two potential mechanisms, shared floral resources and consumption of infected prey, that may explain pathogen transmission between bees and ants. Ants may serve as a previously undescribed reservoir for *Nosema* and *Crithidia* and future research should define the potential for pathogen spillback from ants back into bee populations.

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Chapter 2

Chapter 1: Unifying themes of community ecology and disease ecology through the study of Hymenoptera

Introduction

Emerging infectious diseases are one of the greatest threats contributing to biodiversity loss among wildlife (Daszak et al. 2000). Wildlife diseases affect not only individual hosts, but also populations, communities, and ecosystems (Tompkins et al. 2011). Regardless of the biological scale at which disease dynamics are examined, a key goal of disease ecology involves describing disease prevalence (i.e., number or proportion of individuals with the disease of interest) through space and time (Cooch et al. 2012). To date, most research investigates how diseases impact individual host survival and shape population dynamics with various examples from birds (e.g., George et al. 2015), rodents (e.g., Telfer et al. 2002), and bees (e.g., Betti et al. 2014). However, understanding how disease dynamics in individuals and populations shape disease outcomes for ecological communities remains a challenge (Johnson et al. 2015).

Biodiversity and disease prevalence are likely intrinsically linked—such that higher diversity begets reductions in disease prevalence for communities, which is known as the dilution effect (Johnson and Thieltges, 2010). One mechanism for the dilution effect posits that diversity dilutes disease mainly through the support of species interactions which diminish the most suitable niches for transmission among communities (Johnson et al. 2013a). Hence, when there are increases in host richness and parasite richness for communities, the likelihood that the most virulent parasite will encounter the most transmissible host decreases (Johnson et al. 2013b). Research into

biodiversity-disease relationships have not yet placed these findings into a global change context. One critique is that communities are already undergoing biodiversity loss. Therefore, communities with declining biodiversity are not able to lessen disease transmission through the dilution effect or similar mechanism. Since communities are likely experiencing biodiversity loss already, it is important to understand disease dynamics through a more realistic global change lens. For example, bee communities in Carlinville, Illinois, have already experienced substantial biodiversity losses in the past 100 years (Burkle et al. 2013) and it would be erroneous to assume that these communities have enough host and parasite richness to reduce the probability of the most virulent parasite and the most competent host from interacting. Surveying additional communities may glean further insight into biodiversity-disease relationships and collections of bee communities at Motte Rimrock Reserve (Perris, California) have revealed stable biodiversity since 2014 (Wilson-Rankin, *unpublished data*). There is, however, insufficient data to understand whether diversity among bee communities at Motte Rimrock Reserve have declined or remained stable prior to 2014 making it difficult to directly link this community to Carlinville, Illinois. Still, the stability in diversity that bee communities at Motte Rimrock Reserve have exhibited since 2014 hints that there may potentially be enough host and parasite richness to corroborate the results of Johnson et al. (2013a) and Johnson et al. (2013b).

Bees provide an excellent system to investigate host-pathogen communities compared to other animal models since they have rich assemblages where multiple species can co-occur and these species can be sampled through space and time

(McMahon et al. 2018). Yet, honey bee and wild bee populations are experiencing substantial declines globally (Tylianakis et al. 2013), in part due to the modification of natural habitat from agriculture (Kremen et al. 2002), urbanization (Cardoso & Gonçalves, 2018), pesticides (Goulson, 2013), climate change (Soroye et al. 2020), invasive species (Morales et al. 2013), and pathogens (Fürst et al. 2014). While the plight of pollinators globally in recent decades has prompted numerous investigations into the potential drivers of documented declines (Potts et al. 2010), gut-inhabiting pathogens rank high among the numerous threats to pollinator species (Goulson et al. 2015). Pathogen spillover from managed to wild bee species has been previously documented (e.g., Fürst et al. 2014). Even though pathogen spillover from managed bees has been widely attributed to negative effects for wild bees, quantitative assessments directly linking spillover to negative effects has been vastly underreported (Mallinger, Gaines-Day, & Gratton, 2017). Furthermore, our current understanding of pathogens among wild species has not kept pace with managed species, although recent studies have shed light into the surprisingly high prevalence of pathogens among these wild species (Graystock et al. 2020). One proposed mechanism for pathogen transmission can occur when an infected bee defecates on a flower (Bodden et al. 2019), inoculating the nectar or other floral anatomy such as the bracts (Figueroa et al. 2019), which may potentially infect subsequent bees foraging on that flower (Graystock et al. 2015). This mode of fecal-oral disease transmission may explain in part pathogen spillover from managed to wild bee species (Graystock et al. 2016).

If flowers are common hubs of pathogen transmission among bees, then it is important to determine the role of non-bee floral visitors in structuring transmission dynamics. Ants are aggressive nectarivores (Haber et al. 1981), but they can also be effective pollinators (Del-Claro et al. 2019). There have also been studies which have documented interspecific interactions among bees and ants at flowers. Bees can be harassed by ants at floral resources (Sidhu & Wilson Rankin, 2016) and ants affect floral visitation behaviors of bees such that less effective pollination services may be delivered (Unni et al. 2021). Pathogen spillover may be common between bees and another major floral visitor, ants (Sébastien et al. 2015; Lester et al. 2019). Yet, the link between floral visitation patterns of bees and ants in shaping pathogen community dynamics has not been made. Such findings could explain how pathogen spillover may occur between these taxa and could provide further insights as to how host-parasite interactions are structured for hymenopteran communities (Figure 1.1).

Here, we investigated the ecological factors shaping disease patterns for bees and ants. In Chapter 2, we examined pathogen prevalence for bees and ants during six sampling periods, to understand disease patterns at various times of the year, and across nine different sampling locations, to understand variation in pathogen prevalence across the landscape, at an ecological reserve encompassing coastal sage scrub habitat in Southern California. The research objectives from this chapter were to (1) quantify *Nosema* and *Crithidia* prevalence for bees and ants; and (2) assess how *Nosema* and *Crithidia* prevalence patterns change across space and time. In Chapter 3, we contextualized the findings from Chapter 2 and provide insights into two potential

pathogen transmission mechanisms (shared flowers and consumption of infected prey) which offer exciting new avenues of research to pursue in the future.

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Figures

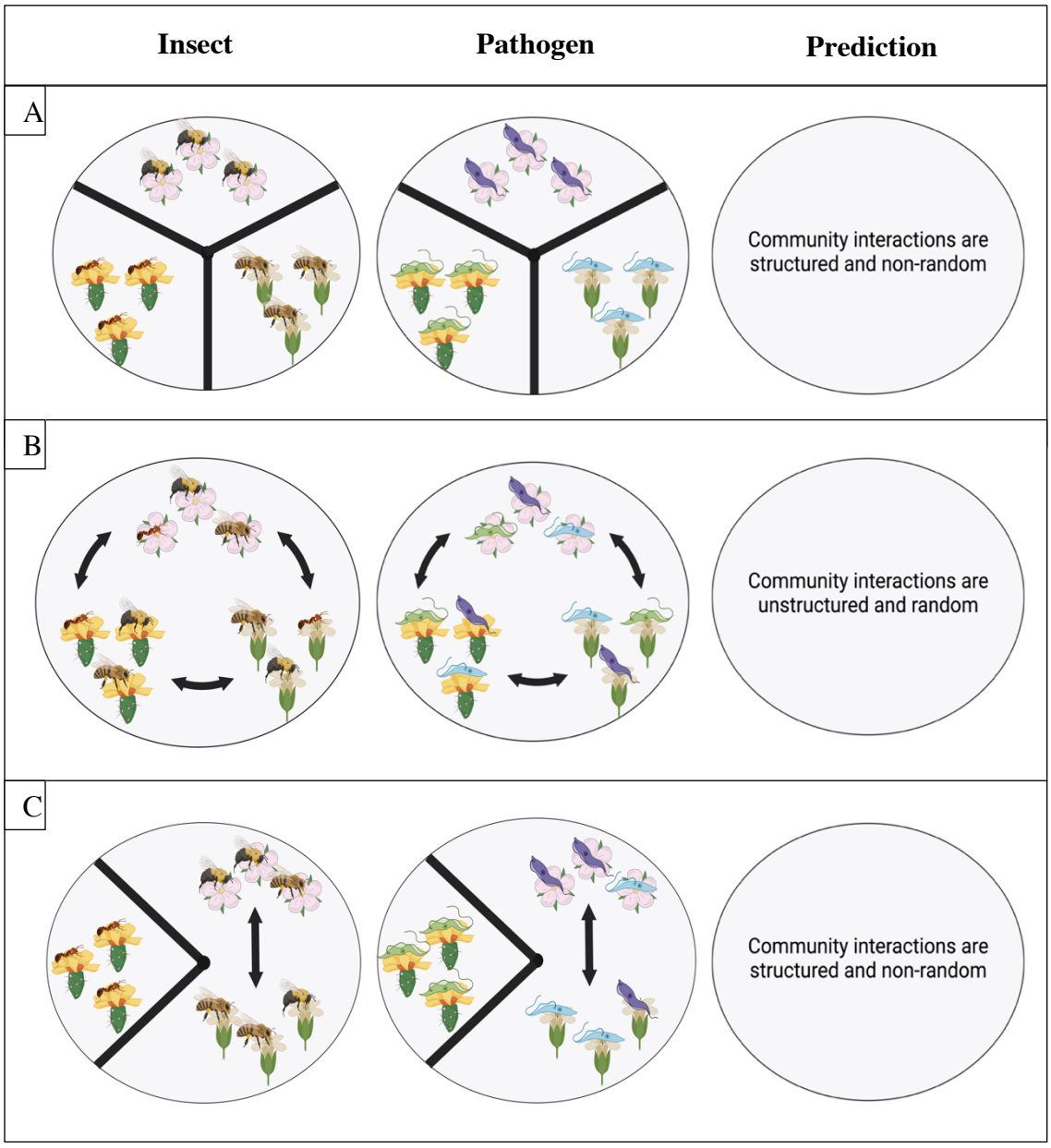
Insect
Pathogen
Prediction
A
Community interactions are structured and non-random
B
Community interactions are unstructured and random
C
Community interactions are structured and non-random

Figure 1.1: There are a multitude of mechanisms to explain how pathogen transmission and spillover might occur at floral resources among hymenopteran communities. A) Each species forages on one resource, promoting intraspecific transmission while inhibiting interspecific transmission. In this community, the observed species interactions would be structured non-randomly and there should be an observed host-pathogen association based on floral visitation patterns. B) Each species forages on each resource, promoting both intra- and interspecific transmission. In this community, the observed species interactions would be randomly structured with no apparent patterns and there would be no host-pathogen association based on floral visitation patterns. C) There is only one species foraging on a particular resource, and the other two species forage on the other two resources. Pathogens could be transmitted interspecifically between the two species sharing resources, but there would not be interspecific transmission from the one species foraging on the other resource. This would result in a predictable outcome for the pathogen community based on floral visitation patterns even if the community is not fully compartmentalizing resources such as in panel A. Floral visitors are indicated by pictures of an ant, honey bee, and bumble bee (representative for all native bees). Pathogens are indicated by green, purple, and blue trypanosome cells. Different flower colors indicate different flower species.

Chapter 2: *Nosema* and *Crithidia* prevalence among bees and ants at Motte Rimrock Reserve (Perris, CA)

Introduction

In the 1980s and 1990s, breakthroughs in ecological theory were challenging the conventional wisdom about plant-pollinator interactions. Researchers demonstrated that these species relationships were not purely mutualistic, rather there were costs for both the plant (Jennersten, 1988) and pollinator (Imhoof & Schmid-Hempel, 1999). The first account of pathogen transmission among bees at shared flowers demonstrated that bumble bees could obtain novel *Crithidia bombi* infections from *Echium vulgare* visitation (Durrer & Schmid-Hempel, 1994). Earlier works prior to Durrer & Schmid-Hempel (1994) hypothesized that social living should be conducive to pathogen transmission (Hamilton, 1964), and therefore focused on the mechanisms, such as polyandry, that would explain how social Hymenoptera minimize pathogen spread among colony members (Sherman, Seeley, & Reeve, 1988). The discovery that flowers were hubs of pathogens highlighted an important aspect of these parasitic organisms, transmission to new hosts was occurring outside of the colony, which appeared to be pervasive across the environment for bumble bees (Imhoof & Schmid-Hempel, 1999). Thus, flower-mediated horizontal transmission opened the door to understanding pathogen dynamics beyond the colony and into the species interactions occurring at flowers. However, the work of Durrer & Schmid-Hempel (1994) did not receive much attention at the time of publication and it was more than a decade later before floral transmission would pique the interest of researchers.

Even before the work of Durrer & Schmid-Hempel (1994), there has long been an interest in the host range of various pathogens among Hymenoptera. Bee parasites, such as *Nosema apis*, have long been known to infect a broad diversity of insects (Fantham & Porter, 1913). In fact, Fantham & Porter (1913) described numerous Hymenoptera including bumble bees, mason bees, and wasps, in which active *N. apis* replication occurred in their food canals. Studies have since made the distinction between *N. apis* and *N. bombi*, showing that these two *Nosema* species are native hosts to honey bees or bumble bees, respectively (Eijnde & Vette, 1993). However, early experiments with *C. mellificae* and *C. bombi* demonstrated an inability for these parasites to be infective beyond the native host (Ruiz-González & Brown, 2006). Even though it could not be demonstrated that *C. bombi* could establish an infection within honey bees, it did appear that honey bees could serve as potential non-host vectors for this parasite (Ruiz-González & Brown, 2006). Thus, indirect species interactions, such as those between honey bees and bumble bees foraging on the same floral resources, may lead to circumstances in which honey bees facilitate pathogen transmission among bumble bees. Such findings encouraged scientists to explore ways in which honey bees and bumble bees were interacting with one another in the environment—which built the foundation of literature which sought to understand the host range of pathogens more broadly across bee species.

As the literature began to expand on pathogen transmission and host specificity in honey bees and bumble bees, there was growing concern over declines in managed honey bee colonies due to colony collapse disorder (CCD) (Oldroyd, 2007). Initial investigations of CCD-positive and CCD-negative colonies found a strong association

between this phenomenon and the presence of *Israeli acute paralysis virus* (IAPV), and while *N. apis* was also found to be a candidate pathogen, there was less overall support compared to IAPV (Cox-Foster et al. 2007). However, the conclusions drawn from Cox-Foster et al. 2007 have been called into question—particularly the claims of Australian imported honey bees leading to pathogen spillover in the US (Anderson & East, 2008). Attention quickly shifted from *N. apis* to the threat of *N. ceranae*, as it increased in frequency over time relative to *N. apis* (Paxton et al. 2007) to become the most widespread *Nosema* species—likely corresponding to increased movement of hives due to increased agricultural demands (Klee et al. 2007). Colonies infected with *N. ceranae* have been shown to collapse when the queen is no longer able to replace the loss in workers (Higes et al. 2008). At the same time, commercially reared colonies of *Bombus occidentalis* were experiencing widespread decline, with *N. bombi* being highlighted as a potential culprit (Velthuis & Van Doorn, 2006). The concurrent decline in wild *B. occidentalis* (Colla & Ratti, 2010) was linked to pathogen spillover from managed to wild *Bombus* populations. For example, a higher prevalence of *C. bombi* and *N. bombi* was documented among wild bumble bees in closer proximity to sites where managed bumble bees were present than those more distant to managed bumble bees (Colla *et al*. 2006). Thus, the threat of pathogen spillover was not only important from a management perspective, but from a biodiversity perspective as well.

Intraspecific pathogen spillover from managed to wild populations is a threat to economically and ecologically important species, such as honey bees and bumble bees. Yet, with ~20,000 bee species found globally (Michener, 2007) and 1,600 occurring in

California alone (Frankie et al. 2009), interest in documenting interspecific pathogen spillover events increased. Such efforts are critical to the conservation of bee species beyond those that are intensively managed. To date, pathogen spillover studies have focused on social bees (honey bees and bumble bees), although several solitary species such as *Megachile rotundata* (Pitts-Singer & Cane, 2011), *Osmia lignaria* (Bosch & Kemp, 2000), and *Nomia melanderi* (Cane, 2008) are economically important species managed for crop production. Yet, the occurrence of traditionally perceived honey bee pathogens, such as *deformed wing virus* (DWV) and *N. ceranae* among wild bumble bees (Fürst et al. 2014), paved the way for further wild bee focused studies. With increased attention and investigations, the number of species in which actively replicated viruses were detected grew from a handful (Singh 2011) to a much wider array of species across the entire order of Hymenoptera (Manley, Boots, & Wilfert, 2015). Molecular detection of negative-strand RNA made determining the host range of viruses a relatively straightforward endeavor (Levitt et al. 2013). Challenges remained when it came to determining the host range of non-viral pathogens, such as *Nosema* and *Crithidia*, because molecular detection of these pathogens only suggest their presence, not active replication. Advances have been made, despite challenges, towards determining the host range of *Crithidia* for solitary bee species such as *M. rotundata*, *O. lignaria*, and *Halictus ligatus*, where cell replication has been demonstrated after pathogen acquisition (Ngor et al. 2020). Studies of wild collected solitary bees have documented *N. ceranae* among genera such as *Osmia* and *Andrena* (Ravoet et al. 2014), which has only added to our overall understanding of non-viral pathogen detection among wild bees. Still, confidently

attributing the detection of non-viral pathogens (e.g., *Nosema* and *Crithidia*) among wild bees to the true host range for these pathogens remains a present challenge.

Another challenge with assessing interspecific pathogen transmission is understanding the diversity of non-bee Hymenoptera, such as wasps and ants, that are potentially involved. The number of studies documenting pathogen spillover among non-bee Hymenoptera has steadily increased between 2010-2020 (Nanetti et al. 2021). Some of the initial reports of pathogen detection among wasps came from *Vespula vulgaris*, which were found to be positive for DWV and *Nosema* (Evison et al. 2012). Curiously, invasive yellowjackets (*Vespula pensylvanica*) in Hawaii experienced a reduction in DWV variant diversity after the introduction of *Varroa* mites – a pattern that was also observed in sympatric honey bees (Loope at al. 2019). Invasive Argentine ants (*Linepithema humile*) appear to also share DWV variants with honey bees in New Zealand (Sébastien et al. 2015; Lester et al. 2019). While there is evidence of viral spillover to non-bee Hymenoptera, there are fewer instances of potential spillover of non-viral pathogens among wasps and no evidence that *Nosema* and *Crithidia* have been detected among ants (Nanetti et al. 2021). The paucity of *Nosema* and *Crithidia* detection among ants represents a clear gap in our current knowledge and determining whether these pathogens are being detected among ants may elucidate previously undescribed pathogen prevalence and transmission among hymenopteran communities.

Here, the goal is to address several knowledge gaps in potential interspecific *Nosema* and *Crithidia* prevalence among hymenopterans. Bees and ants ecologically overlap to some degree as they both utilize floral resources. Because flowers may serve

as a hub of *Nosema* and *Crithidia* transmission among wild bees (Graystock et al. 2020), it is plausible that ants may acquire *Nosema* and *Crithidia* from the environment. While our knowledge of wild bee species potentially implicated in *Nosema* and *Crithidia* transmission is continuously expanding (Figueroa et al. 2020), additional studies can provide an opportunity to corroborate previous findings and document novel pathogen detections among wild bees. We tested these ideas by collecting bees and ants to understand potential variation in *Nosema* and *Crithidia* across space and time—as these factors are central to defining disease patterns among ecological communities (Johnson et al. 2015). *Nosema* and *Crithidia* being detected among bees and ants inhabiting the same space at the same time would suggest that interspecific pathogen transmission may be occurring. This research builds upon our current knowledge of Hymenoptera that may be implicated in the exposure, acquisition, and spread of *Nosema* and *Crithidia*.

Research Objectives

1) Quantify *Nosema* and *Crithidia* prevalence for bees and ants.
2) Assess how *Nosema* and *Crithidia* prevalence patterns change across space and time.

Methods

Study Site

Motte Rimrock Reserve (Perris, CA) is an ecological reserve in Southern California dominated by charismatic coastal sage scrub habitat. Coastal sage scrub represents one of the most threatened native plant communities in the United States (Burger et al. 2003). Social insects, such as honey bees and ants, are highly prominent at

Motte Rimrock Reserve (Sidhu & Wilson Rankin, 2015). Because social species tend to be more numerous across the landscape (e.g., Cecala & Wilson Rankin, 2021), social species at Motte Rimrock Reserve may increase the rate of intraspecific pathogen transmission as has been demonstrated in other bee systems (Yoneda et al. 2008), which may influence pathogen patterns for hymenopteran communities at Motte Rimrock Reserve.

Insect Sampling and Identification

At Motte Rimrock Reserve, we monitored the local prevalence of bee pathogens, *Nosema* and *Crithidia,* in floral visitors across the year. We sampled bees and ants during 6 seasonal periods that were biologically reflective of distinct transitions throughout the year: early spring, mid spring, and late spring—when local temperatures and floral resources steadily increase; midsummer and late summer—when local temperatures are at their peak and all spring blooms have fully senesced; mid fall—when local temperatures substantially cool off and the last blooms of the year begin to senesce. Midsummer, late summer, and mid fall samplings occurred in 2019 while early spring, mid spring, and late spring samplings took place in 2020 ('sampling period'). At 9 spatially separate sites throughout Motte Rimrock Reserve (Figure 2.1), we established 100-m sampling transects ('sampling location'). These sites were selected because they were >150m from one another and encompassed various dominant flowering plant species while also hosting a broad diversity of species that are characteristic of coastal sage scrub ecosystems (CWA, *pers. obs.*). Along each transect during each sampling period, two collectors sampled bees from flowers via sweep netting during 15-minute

timed collections (N = 27 hours of sampling effort during study; n = 4.5 hours of sampling effort per sampling period). To collect ants, 2 grams of Keebler® Sandies® Pecan Shortbread Cookies were placed on a white note card. Note cards were evenly spaced every 25m along transects (n = 4 per transect) and were collected after 60 minutes (*sensu* Wetterer et al. 2001) from each sampling location during each sampling period (N = 216 hours of sampling effort during study; n = 36 hours of sampling effort per sampling period). All collected insect samples were placed in a liquid nitrogen dry shipper until they were brought back to the lab and then frozen at -30 °C until further processing. Bee species identifications were performed or confirmed by the University of California, Riverside Senior Museum Scientist Dr. Doug Yanega, often making use of synoptic material in the UCR Entomology Research Museum. We identified all wild bees to species (or genus when specimens could not be confidently assigned to species) and sex-assigned bees when possible. Ant species identifications were performed by lab personnel with previous training in ant identification.

Pathogen Detection

In preparation for pathogen analysis, all collected specimens were mixed with 70% ethanol to wash away pathogens on the surface and dried at room temperature for 1 minute. We pooled together individual ants from the same note card that were collected from the same sampling period and sampling location (n = 20 ants per pool) for subsequent pathogen screening. Individual bees had abdomens removed, containing both the midguts and hindguts as this is where *Nosema* and *Crithidia* reside, respectively. Whole bodies of ants were homogenized. DNA was extracted from these samples using a

DNeasy Blood & Tissue Kit (Qiagen) following manufacturer protocols. Samples were not lysed with a bead beating protocol (*sensu* Rubanov et al. 2019) prior to extraction. We screened these extracts for *Nosema* using primers *V1f* and *530r* (Baker et al. 1995) and for *Crithidia* using *CB-SSUrRNA-F2* and *CB-SSUrRNA-B4* (Schmid-Hempel and Tognazzo 2010). To confirm our screens were working, we amplified the COI gene for a subset of samples (which served as a positive control) and included a negative control (no DNA sample included) to ensure the absence of contamination. A multi-plex PCR reaction volume of 15 μL comprised the following: 2 μL of template DNA, 3 μL of 5x GoTaq Reaction Buffer, 1.2 μL of 15mM MgCl2, 1.2 μL 1X BSA, 0.75 μL each of V1f/530r primers (10nmol), 0.75 μL each of *CB-SSUrRNA-F2*/*CB-SSUrRNA-B4* primers (10nmol), 0.1 μL GoTaq polymerase, 1.2 μL of dNTPs (2.5mM), and sterile molecular-grade water. PCR amplification was then performed under the following reaction conditions: an initial denaturation step of 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 60 s. A final extension step was performed at 72°C for 5 min. Presence of pathogens was then determined by the presence of bands on a 1.5% agarose gel. A subset of PCR products was Sanger sequenced to confirm the amplification of *Nosema* and *Crithidia*, respectively.

Statistical Analyses

All analyses were performed in R version 4.0.3 (R Core Team 2020). We performed a series of logistic regression generalized linear models (GLM) using the function *glm* (package 'lmerTest') (Kuznetsova et al. 2017) to assess what ecological

factors were associated with the presence of *Nosema* or *Crithidia* in individual bees and pooled ants. We ran separate GLMs for each pathogen, using presence (yes/no) as the dependent variable with a quasibinomial error structure with a logit link to correct for overdispersion in the data, where sampling period, sampling location, and insect taxa (honey bees, native bees, and ants) were included as predictor variables. While there is no threshold to determine when overdispersion should be corrected for (Payne et al. 2018), we feel confident in our decision to use a quasibinomial versus a binomial error structure in our GLMs since model summaries indicated a dispersion parameter >1 with a binomial error structure and <1 with a quasibinomial error structure. We also tested for interactions among insect taxa, sampling period, and sampling location. When appropriate, we conducted *post hoc* tests using the function *glht* (package 'multcomp') (Hothorn et al. 2008) correcting for multiple comparisons using the false discovery rate. In several cases, our *post hoc* analyses did not reveal significant differences among levels of a factor, although there was a significant effect. This was due in part to small sample sizes for some categories and complete separation of the data (no variance) in other categories (see Discussion). Overall, this set of analyses allowed us to understand whether main effects of sampling period, sampling location, or insect taxa were shaping the prevalence of *Nosema* or *Crithidia*. Furthermore, testing for interactions allowed us to understand whether pathogen prevalence for a given insect taxa was dependent upon the sampling period or sampling location being considered.

Results

A total of 443 bees were collected during the study representing 26 genera from 5 families. Of that total, 241 were honey bees (*Apis mellifera*), which were most likely locally established feral colonies within or surrounding Motte Rimrock Reserve. The remaining 202 samples comprise various native bee species (Table 2.1). Of the bees collected, 385 were female and 77 were male. Collectively, honey bees and native bees visited 34 different plant species (Table 2.2), representing 16 families, accounting for 109 unique plant host and bee genus interactions. We collected 380 ant specimens, of which 200 were identified as *Forelius mccooki* and the other 180 were *Solenopsis xyloni*, resulting in 19 pooled samples for pathogen screens.

Spatial and temporal differences in species richness were observed for bees and ants. There was considerably less variation in the number of samples collected from each sampling location (mean ± SE = 51.33 ± 3.10 samples per location) compared to sampling period (mean ± SE = 73.17 ± 17.76 samples per sampling period). A total of 14 bee species were collected at site 'SQU', making it the most species rich sampling location. Only 7 bee species were detected at site 'GOL', making it the least species rich sampling location. On average, 11 ± 0.73 (mean ± SE) bee species were collected from each sampling location. Across sampling periods, 16 bee species were collected during mid spring, making it the most species rich sampling period. Conversely, only 4 bee species were collected during mid fall, making it the least species rich sampling period. An average of 10 ± 1.65 (mean ± SE) species were collected from each sampling period. For ants, *F. mccooki* was the only species collected from sampling locations "BRO',

'BUC', and 'SAG'. Both *F. mccooki* and *S. xyloni* were collected from sampling locations 'CAC', 'GOL', 'HIL', and 'SQU'. We did not detect any ants at sampling locations 'ANN' or 'POP'. Temporally, *F. mccooki* was the only species detected during late summer and *S. xyloni* was the only species detected during mid fall and early spring. Ants were not detected during midsummer, mid spring, and late spring.

In all, 462 *Nosema* and *Crithidia* screens were conducted. Of those screens, 342 were negative for both *Nosema* and *Crithidia*, 20 were positive for both *Nosema* and *Crithidia*, 22 were negative for *Nosema* and positive for *Crithidia*, and 78 were positive for *Nosema* and negative for *Crithidia* (Table 2.1). *Nosema* was detected in all 5 bee families collected: Andrenidae, Apidae, Halictidae, Megachilidae, and Melittidae. Halictidae was the most common bee family collected during this study when excluding *Apis* from Apidae. *Nosema* was detected in most Halictidae genera (7/8). We only collected 4 samples from the family Melittidae, all belonging to the genus *Hesperapis*, yet the majority (3/4) screened positive for *Nosema*. *Crithidia* was only detected in three bee families: Andrenidae, Apidae, and Halictidae. *Crithidia* occurred most frequently among the genera of Apidae (4/10; including *Apis*) compared to Andrenidae (1/4) and Halictidae (1/8). *Nosema* and *Crithidia* were co-detected (presence of both pathogens in a sample) in both Apidae and Halictidae but not Andrenidae. We did not examine the relationship between pathogen prevalence and plant species due to collinearity. Although, qualitatively, bees visiting plants in the family Boraginaceae frequently tested positive for *Nosema* (6/6 plants had at least 1 bee screen positive; see Table 2.2). There was no discernable association between plant family and *Crithidia* prevalence. Among the ant

pathogen screens, all *F. mccooki* screens were positive for *Crithidia* (n =10), with half also screening positive for *Nosema*. The majority of *S. xyloni* screens were negative for both pathogens (n = 6), with only a few screens positive for either *Nosema* (n = 2) or *Crithidia* (n = 1).

A qualitative assessment revealed that there were distinct pathogen prevalence patterns for honey bees and native bees across the landscape (Figure 2.2). Honey bees were positive for *Crithidia* across all sampling locations and were positive for *Nosema* at most sites (7/9). While *Crithidia* was more consistently detected across the landscape among honey bees, when *Nosema* was present at a sampling location it was typically more prevalent than *Crithidia*. Similar patterns for *Nosema* prevalence were observed among native bees as well. *Nosema* was detected at all sampling locations for native bees and was more prevalent than *Crithidia* at most sampling locations (8/9). Comparing the proportion of honey bee and native bee pathogen positive (i.e., *Nosema* positive only, *Crithidia* positive only, and *Nosema* + *Crithidia* co-detection) to pathogen negative (i.e., *Crithidia* and *Nosema* were 'undetected') samples across the landscape revealed that there was more pathogen negative than pathogen positive samples in all instances except one (native bees at site 'BUC').

Nosema prevalence was influenced by sampling period ($\chi^2_5 = 39.11$; $p < 0.001$) and sampling location ($\chi^2_8 = 16.99$; $p = 0.03$). However, no differences were detected during subsequent *post hoc* analyses due to complete data separation (see Discussion). Furthermore, there was no main effect of insect taxa (i.e., honey bees, native bees, and ants) on *Nosema* prevalence ($\chi^2_2 = 4.43$; $p = 0.11$). There was a significant sampling

period × insect taxa interaction ($\chi^2_7 = 22.51$; $p = 0.002$; Figure 2.3), such that honey bees in midsummer had a substantially lower prevalence of *Nosema* than: honey bees in late summer ($z = -4.02$; $p = 0.002$) mid spring ($z = -3.88$; $p = 0.002$), and late spring ($z = -3.05$; $p = 0.02$), native bees in midsummer ($z = 3.11$; $p = 0.02$), late summer ($z = -4.8$; $p < 0.001$), early spring ($z = -3.79$; $p = 0.003$), mid spring ($z = -6.33$; $p < 0.001$), and late spring ($z = -3.08$; $p = 0.02$), and ants in late summer ($z = -4.09$; $p = 0.001$). Additionally, native bees in mid spring had a higher prevalence of *Nosema* than native bees in late spring ($z = 3.32$; $p = 0.01$). We observed separation in the data, in which all *Nosema* screens for native bees during mid fall ($n = 14$) and all pooled ants during early spring ($n = 4$) were negative, resulting in no variation for these groups (SE = 0). There was also a significant sampling location × insect taxa interaction ($\chi^2_{14} = 30.79$; $p = 0.005$; Figure 2.4), yet no differences were detected upon subsequent *post hoc* analysis. Again, we observed separation in the data, in which all *Nosema* screens for ants at sampling locations 'SQU' ($n = 3$), 'CAC' ($n = 2$), 'HIL' ($n = 3$), and all screens for honey bees at 'BRO' ($n = 18$) were negative, resulting in no variation for these groups (SE = 0). Additionally, we observed separation in which all *Nosema* screens for ants were positive at sampling location 'BRO' ($n = 2$), resulting in no variation for this group (SE = 0). Furthermore, we detected a significant sampling location × sampling period interaction ($\chi^2_{40} = 93.55$; $p < 0.001$), yet there were no differences detected upon subsequent *post hoc* analysis.

Crithidia prevalence was influenced by sampling period ($\chi^2_5 = 126.57$; $p < 0.001$) and insect taxa ($\chi^2_2 = 89.35$; $p < 0.001$), but not sampling location ($\chi^2_8 = 7.42$; $p = 0.49$).

There were no differences detected upon subsequent *post hoc* analyses for sampling period and insect taxa. There was a significant sampling period × insect taxa interaction ($\chi^2_7 = 76.29$; $p < 0.001$; Figure 2.5), such that honey bees in midsummer had a lower prevalence of *Crithidia* than honey bees in late summer ($z = -6.26$; $p < 0.001$), mid fall ($z = -3.44$; $p = 0.008$), and late spring ($z = -3.92$; $p = 0.002$). Furthermore, honey bees in late summer had a higher prevalence of *Crithidia* than: honey bees in mid fall ($z = 3.21$; $p = 0.02$), early spring ($z = 3.33$; $p = 0.01$), mid spring ($z = 3.46$; $p = 0.008$) and late spring ($z = 3.18$; $p = 0.02$), and native bees in midsummer ($z = -2.94$; $p = 0.03$), late summer ($z = 3.99$; $p = 0.002$), early spring ($z = 3.95$; $p = 0.002$), and mid spring ($z = 4.66$; $p < 0.001$). We observed complete data separation in which all *Crithidia* screens for native bees in mid fall ($n = 14$) and late spring ($n = 58$) and all ants in early spring ($n = 4$) were negative, resulting in no variation for these groups (SE = 0). Conversely, we detected separation in which all *Crithidia* screens for ants in the late summer ($n = 10$) were positive, resulting in no variation for this group (SE = 0). There was also a significant sampling location × insect taxa interaction ($\chi^2_{14} = 47.56$; $p < 0.001$; Figure 2.6), yet no differences were detected upon subsequent *post hoc* analysis. Again, we observed separation in which all *Crithidia* screens for native bees at sampling locations 'CAC' ($n = 26$), 'GOL' ($n = 12$), and 'BUC' ($n = 13$) were negative, resulting in no variation for these groups (SE = 0). On the other hand, we observed separation in which all *Crithidia* screens for ants at sampling locations "CAC' ($n = 2$) and 'BRO' ($n = 2$) were positive, resulting in no variation for these groups (SE = 0). Furthermore, we detected a significant sampling location × sampling period interaction ($\chi^2_{40} = 155.09$; $p < 0.001$), yet there were

no differences detected upon subsequent *post hoc* analysis due to complete data separation.

Discussion

To our knowledge, *Nosema* and *Crithidia* have not been previously reported for several native bee genera: *Calliopsis*, *Conanthalictus*, *Diadasia, Dufourea, Habropoda, Hesperapis, Perdita,* and *Xenoglossa*. Additionally, our findings corroborate reports of *Nosema* and *Crithidia* for several native bee genera: *Agapostemon*, *Andrena*, *Anthophora*, *Augochlorella, Ceratina, Halictus, Hoplitis, Megachile, Lasioglossum,* and *Osmia* (Figueroa et al. 2020; Graystock et al. 2020). We have also provided the first description, to our knowledge, of *Nosema* and *Crithidia* detection among ants in general. More specifically, this is the first description of "bee" pathogens being detected in *Forelius mccooki* and *Solenopsis xyloni* – two native ant species in Southern California.

Native bees were more commonly detected with *Nosema* compared to *Crithidia*, and this trend was consistent for both sampling location and period. *Nosema* appeared to be most prevalent at sampling location, 'BUC', which was dominated by California buckwheat (*Eriogonum fasciculatum*). Potentially, there are features present in the landscape at this location which were conducive to increasing *Nosema* prevalence, such as the dominance of specific plant species. In anthropogenically modified landscapes, it does not appear that the specific foraging patterns of bees was indicative of pathogen prevalence, but rather bees foraging in landscapes with a greater proportion of simplified, low diversity, monoculture cover shaped pathogen prevalence (Figueroa et al. 2020). Perhaps, in natural systems, the dominance of specific plant species, such as California

buckwheat, creates similar low diversity conditions observed with monocultures. Although, at Motte Rimrock Reserve, California buckwheat puts on an impressive and highly abundant floral display when in full bloom (CWA, *pers. obs.*). Increases in floral abundance may decrease pathogen prevalence as has been shown in natural sites in upstate New York (Graystock et al. 2020). It may be important in the future to further investigate floral resource diversity and bee visitation patterns across natural landscapes to see whether this plays a role in pathogen prevalence, which, curiously, would also provide further evidence for the dilution effect hypothesis.

Temporally, it is interesting that there was a significant decrease in the prevalence of *Nosema* among native bees from mid spring to late spring. Mid spring also corresponds to the season which had the highest species richness for both bees and plants (16 species each), which seems to contradict the dilution effect hypothesis. Potentially, there is a time delay in which the conditions of one sampling period shape the observed pathogen patterns in the following sampling period. For example, there were 11 bee species and 5 plant species in early spring compared to 15 bee species and 16 plant species in mid spring. While lower plant and bee species richness was observed during early spring, it was also the only sampling period with a greater number of male bees collected ($n = 20$) than female bees ($n = 12$). Although this was not a specific aim of the study to discern pathogen differences among male and female bees, it has been previously demonstrated that male *Osmia cornuta*, but not females, seem to be more susceptible to *C. mellificae* exposure (Strobl et al. 2019). Additionally, male honey bees, which were not collected during this study, have been shown to be more susceptible to *N.*

ceranae infection (Retschnig et al. 2014). There is also a time delay from the initial acquisition of *Nosema* and *Crithidia* to when they reach peak-infection levels within the host (see Ngor et al. 2020 and Figueroa et al. 2021 for peak infection times). Possibly, the peak in *Nosema* prevalence observed in mid spring is a lagging result from the conditions observed in early spring such as when plant and bee diversity are low and pathogen susceptibility among individuals is high (potentially from a high number of males). A delayed response mechanism shaping pathogen prevalence would therefore still be consistent with rather than contradict the dilution effect hypothesis.

Ants exhibited higher *Nosema* prevalence in the late summer compared to honey bees in midsummer, although the prevalence of *Nosema* did not differ among any insect taxa during late summer. There are other genera of microsporidia, such as *Thelohania solenopsae* and *Vairimorpha invictae*, which can have substantial effects on the fitness of red imported fire ants (*Solenopsis invicta*) (Williams et al. 1999; Oi et al. 2005). As some microsporidia are known to have such detrimental effects on *Solenopsis*, future studies could quantify how *Nosema* species affect fire ant fitness, such as *S. xyloni* which was collected during this study, if at all. Ants in this study also experienced a spike in *Crithidia* prevalence during the late summer as all pooled pathogen screens during this sampling period were positive for the pathogen. It could not, however, be statistically supported with our current analysis as to whether ant *Crithidia* prevalence in late summer was different from other groups due to data separation. At present, there is no previous documentation of trypanasomatids being detected among ants to our knowledge. The high prevalence of both pathogens in late summer may be at least partially explained by

the observed species turnover between the summer (*Forelius mccooki*) and fall and spring (*Solenopsis xyloni*), such that *F. mccooki* are potentially acquiring *Nosema* and *Crithidia* at higher rates than *S. xyloni*. Our findings of peak pathogen prevalence in the summer are consistent with peaks in the microsporidian, *Kneallhazia solenopsae*, among imported fire ants during warmer months of the year (Valles et al. 2010).

Here, we pooled ants into groups of 20 for pathogen screens. While pooling ants for pathogen screening is an accepted method in the literature (Briano et al. 2006), future studies could focus on increasing the number of colonies sampled or increasing the number of baits laid per location. Moreover, a study on colony-level virus prevalence among yellow crazy ants (*Anoplolepis gracilipes*) revealed that all colonies surveyed in Okinawa and Taiwan were infected (Hsu et al. 2008), which demonstrates that pathogens among ants can be highly prevalent across the landscape. When comparing pathogen patterns between ants and bees though, it may be more informative to screen a combination of whole colonies and individuals (*sensu* Sébastien et al. 2015). A combination of colony-level and individual pathogen screening may be the best approach in future studies, especially when considering that the total sampling effort for ants alone was 216 hours and only yielded 19 pooled screens.

Honey bees (*Apis mellifera*) were the most ubiquitous insect collected during this study – accounting for more than half of the total samples collected. Curiously, honey bees had a lower prevalence of *Nosema* during the midsummer compared to native bees during most sampling periods ($n = 5$). Conversely, honey bees in late summer experienced a spike in *Crithidia* that was markedly higher than *Crithidia* prevalence for

native bees across all sampling periods. It has been previously observed among managed honey bee colonies in Serbia that *N. ceranae* infections decrease in July which corresponds to annual peaks in average temperatures for that region followed by a spike in infection when temperatures begin to drop in August (Vejnovic et al. 2018). These results appear consistent with our findings from a temporal perspective, since we observed low prevalence in midsummer (July), followed by a peak in late summer (August). In Southern California, temperatures are consistently high in July and August and do not begin to noticeably drop until October (NOAA National Centers for Environmental Information). Therefore, in our study system at least, there are likely additional factors beyond temperature that are shaping the observed pathogen patterns. Similarly, in Southern Ontario, Canada, managed honey bee colonies exhibited higher *N. ceranae* infections in spring and summer with a drop in infections in the fall (Emsen et al. 2020). Even though infection levels were similar in spring and summer, spore viability was substantially lower in the summer. While we did observe similar temporal fluctuations as Vejnovic et al. (2018), it may also be important to also consider pathogen viability across sampling periods in the future to see whether they corroborate the findings of Emsen et al. (2020).

The main distinction between Vejnovic et al. (2018) and Emsen et al. (2020) to this study is that we examined temporal pathogen patterns among feral, non-managed honey bees. Studies have documented that Africanized genes are integrated into the feral honey bee populations of Southern California (Kono & Kohn 2015; Cridland et al. 2018). Africanized honey bees can be exceptionally resistant to some parasites (Kraus & Page,

1995). While reduced longevity and alterations in foraging behavior from *N. ceranae* infection has been observed among Africanized honey bees, the severity of these negative effects is moderate compared to European lineages (Fleites-Ayil, Quezada-Euán, & Medina-Medina 2018) and does not appear to result in colony collapse (Guimarães-Cestaro et al. 2020). Yet, the honey bees of Southern California should not be considered fully Africanized, as is the case in regions of the Americas such as Panamá, but rather they are a chimera of African, Western European, Eastern European, and even Middle Eastern ancestries (Zárate et al. 2022). Potentially, this unique admixture produces differential pathogen responses resulting in low *Nosema* prevalence and a higher prevalence, comparatively, for *Crithidia* among honey bees at Motte Rimrock Reserve.

Floral visitation patterns were one factor of particular interest to us since there is a rich history of literature documenting how interactions between bees and plants may be shaping pathogen prevalence (e.g., Durrer & Schmid-Hempel, 1994). While we did document host-associations for both honey bees and native bees, our initial analyses indicated collinearity between plant host and pathogen prevalence in part due to low sample size, and we therefore did not examine the relationship statistically. Collinearity, coupled with low sample size, is indicative of low statistical power which can result in misleading data interpretation since there is an increased probability of Type II error (Mason & Perreault Jr, 1991). Even though there were a total of 34 plant hosts observed during the study, accounting for 109 unique plant host and bee genus interactions; there were only 4 plant host and bee genera interactions that had a sample size >10. A similar pattern was seen for the 33 unique plant host and pathogen combinations observed. Only

two plant host and pathogen combinations had a sample size >10: *Eriogonum fasciculatum* and *Nosema* (n = 16) and *Eriogonum fasciculatum* and *Crithidia* (n = 11). Broadening the taxonomic scale of plants from genus to family increased group sample sizes and we were able to observe that bees visiting plants in the family Boraginaceae consistently screened positive for *Nosema* (Table 2.2). There was no discernable association between plant family and *Crithidia* prevalence in our study though. In the future, a more targeted sampling approach focused on specific flowering taxa in coastal sage scrub ecosystems, such as *Eriogonum fasciculatum* or plants in Boraginaceae, may increase sample sizes which would allow us to examine relationships between plant hosts and floral visitors in greater detail.

While this study has provided greater understanding into the potentially shared pathosphere for *Nosema* and *Crithidia* among hymenopterans, there are potential caveats with the current statistical approach that need to be discussed in further detail before drawing definitive conclusions from our analyses. Separation was observed on 16 occasions during our analyses. In the context of our analyses, data separation likely occurred due to an overspecification of the interaction variables. While separation indicates a perfect prediction of the response, this is considered unrealistic since it implies infinite or zero maximum likelihood estimates of odds ratios (Heinze, 2006). When separation does occur, a sample size >100 is recommended for meaningful, predictive validation (Steyerberg, 2018). There were no groups in our study in which separation occurred that possessed a sample size >100. Alternatively, when a sample size cannot be increased, it is recommended to use penalized-likelihood methods to remove

separation (Mansournia et al. 2018). Still, even with small sample sizes, such observations can provide potentially novel biological insights (Steyerberg, 2018), which is why we chose to include the separated data in our analyses. For example, we encourage future studies to question why *Crithidia* was not detected among any of the native bees sampled during the late spring sampling period; or, why *Crithidia* was not detected among the honey bees at sampling location 'POP'? Hence, while there are limitations to the predictive power of this study, there are exciting, novel questions that still arise such as:

1) Are the *Nosema* and *Crithidia* species detected in ants the same as the ones found in bees?
2) Does the presence of *Nosema* and *Crithidia* in bees or ants result in disease?
3) How widespread are *Nosema* and *Crithidia* among bee and ant species beyond the ones surveyed in this study?
4) What are the potential transmission routes between bees and ants?
5) What are the potential consequences of resulting *Nosema* and *Crithidia* diseases for bee and ant population dynamics?

Evidence to test the first question could be ascertained from samples already collected during this study. Bee and ant pathogen screens could be sequenced and compared against a BLAST search to confirm species identity. Sequences could be further compared by examining regions of base pair dissimilarity to see whether point mutations result in synonymous or non-synonymous amino acid changes. These additional analyses would elucidate how many species of *Nosema* and *Crithidia* there were and how many

variants of each species were present. Phylogenetic approaches could also be used to see if pathogen strains are clustering based on the taxonomic rank of the host (either by family or genera). A recent study of viruses among bees in Southern France found a phylogenetic clustering of *acute bee paralysis viruses* among *Eucera* bees and a clustering of *deformed wing virus* strains among honey bees and bumble bees (Dalmon et al. 2021). Furthermore, reanalyzing the data with a qPCR approach would allow us to quantify the absolute abundance of pathogens among our samples, which may lead to additional spatiotemporal insights such as differences in absolute abundance across sampling locations/sampling periods.

In summary, there are a variety of bee and ant species in which this is the first record of *Nosema* and *Crithidia* detection. Honey bees exhibited substantial fluctuations in the prevalence of *Nosema* and *Crithidia* between sampling periods, which was consistent with previous documentations in the literature. Conversely, the differences across sampling locations were more moderate for honey bees. *Nosema* appeared to be more prevalent among native bees while *Crithidia* was more prevalent among honey bees—a trend that was observed at both spatial and temporal scales. It is unclear at present whether floral visitation patterns are shaping the pathogen outcomes observed for honey bees and native bees. Given, however, the presence of *Nosema* and *Crithidia* among native bees does suggest that acquisition was likely occurring at floral resources. Even though the sample size was low for ants, it is curious that pathogen prevalence was typically all-or-nothing, such that all the screens at a given sampling location or period were positive or none were. While we were most interested in understanding how space

and time were shaping pathogen prevalence among honey bees, native bees, and ants, we also found that there was an interaction between sampling location and sampling period. This finding suggests that there is potentially a wrong place at the wrong time scenario occurring in which certain places at certain times are particularly important for shaping pathogen prevalence, regardless of the insect taxa in question. Collectively, the findings from this study frequently demonstrated the detection of *Nosema* and *Crithidia* among bees and ants, which suggests that there are species interactions occurring in this coastal sage scrub community which are leading to the exposure, acquisition, and transmission of these pathogens.

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Tables

Table 2.1: Pathogen prevalence among bees and ants. Insects are characterized by 'Taxa' ('Ant' or 'Bee'), 'Family', and 'Genus'. The number of samples screened is indicated in column 'Total'. Pathogen screens were separated by the sample size and percentage of samples that either screened positive for both pathogens ('*Crithidia* (+) *Nosema* (+)'), positive for *Crithidia* only ('*Crithidia* (+) *Nosema* (-)'), or positive for *Nosema* only ('*Crithidia* (-) *Nosema* (+)'). Rows with bold text indicate the first record of *Nosema* and/or *Crithidia* detection among the specified taxa.

Taxa	*Family*	*Genus*	*Total*	*Crithidia (+) Nosema (+)*	*Crithidia (+) Nosema (-)*	*Crithidia (-) Nosema (+)*
Ant	**Formicidae**	***Forelius***	**10**	**5 (50%)**	**5 (50%)**	**0**
Ant	**Formicidae**	***Solenopsis***	**9**	**0**	**1 (11.1%)**	**2 (22.2%)**
Bee	Andrenidae	*Andrena*	4	0	0	2 (50%)
Bee	Andrenidae	*Andrena (Diandrena)*	7	0	0	2 (28.6%)
Bee	**Andrenidae**	***Calliopsis***	**3**	**0**	**1 (33.3%)**	**0**
Bee	**Andrenidae**	***Perdita***	**11**	**0**	**0**	**6 (54.6%)**
Bee	Apidae	*Anthophora*	13	1 (7.7%)	1 (7.7%)	2 (15.4%)
Bee	Apidae	*Anthophora (Heliophile)*	8	0	0	3 (37.5%)
Bee	Apidae	*Apis*	241	11 (4.6%)	13 (5.4%)	18 (7.5%)
Bee	Apidae	*Ceratina*	2	1 (50%)	0	1 (50%)
Bee	**Apidae**	***Diadasia***	**18**	**0**	**0**	**2 (11.1%)**

Bee	Apidae	*Eucera*	1	0	0	0
Bee	**Apidae**	***Habropoda***	**8**	**1 (12.5%)**	**1 (12.5%)**	**0**
Bee	Apidae	*Melissodes*	5	0	0	0
Bee	Apidae	*Peponapis*	1	0	0	0
Bee	**Apidae**	***Xenoglossa***	**4**	**0**	**0**	**1 (25%)**
Bee	Halictidae	*Agapostemon*	15	0	0	4 (26.7%)
Bee	Halictidae	*Augochlorella*	10	0	0	3 (30%)
Bee	**Halictidae**	***Conanthalictus***	**7**	**0**	**0**	**3 (42.9%)**
Bee	**Halictidae**	***Dufourea***	**6**	**0**	**0**	**2 (33.3%)**
Bee	Halictidae	*Halictus*	29	1 (3.5%)	0	5 (17.2%)
Bee	Halictidae	*Lasioglossum*	3	0	0	2 (66.7%)
Bee	Halictidae	*Lasioglossum (Dialictus)*	39	0	0	14 (35.9%)
Bee	Halictidae	*Lasioglossum (Evylaeus)*	1	0	0	0
Bee	Megachilidae	*Hoplitis*	8	0	0	4 (50%)
Bee	Megachilidae	*Megachile*	3	0	0	1 (33.3%)
Bee	Megachilidae	*Osmia*	1	0	0	0
Bee	**Melittidae**	***Hesperapis***	**4**	**0**	**0**	**3 (75%)**

Table 2.2: Pathogen prevalence among bees by plant-host association. Plants are separated by 'Family' and 'Species'. The column 'Total Visitation' represents the sample size of individual bees that were screened for pathogens. Pathogen screens were separated by the sample size and percentage of samples that either screened positive for both pathogens ('*Crithidia* (+) *Nosema* (+)'), positive for *Crithidia* only ('*Crithidia* (+) *Nosema* (-)'), or positive for *Nosema* only ('*Crithidia* (-) *Nosema* (+)').

Family	*Species*	*Total Visitation*	*Crithidia (+) Nosema (+)*	*Crithidia (+) Nosema (-)*	*Nosema (+) Crithidia (-)*
Adoxaceae	*Sambucus mexicana*	4	0	0	1 (25%)
Asteraceae	*Corethrogyne filaginifolia*	7	0	0	0
Asteraceae	*Encelia farinosa*	10	0	0	3 (30%)
Asteraceae	*Gutierrezia sarothrae*	17	1 (5.9%)	0	3 (17.7%)
Asteraceae	*Helianthus annuus*	1	0	0	0
Asteraceae	*Lasthenia californica*	14	0	0	6 (42.9%)
Asteraceae	*Oncosiphon pilulifer*	2	0	0	2 (100%)
Asteraceae	*Stephanomeria exigua*	2	0	0	0
Asteraceae	*Stephanomeria virgata*	12	0	0	0
Asteraceae	*Tanacetum* spp.	2	0	0	0
Boraginaceae	*Amsinckia menziesii*	26	3 (11.5%)	2 (7.7%)	1 (3.9%)
Boraginaceae	*Nemophila menziesii*	1	0	0	1 (100%)
Boraginaceae	*Phacelia cicutaria*	4	0	0	2 (50%)

Boraginaceae	*Phacelia distans*	9	0	0	4 (44.4%)
Boraginaceae	*Phacelia ramosissima*	11	0	0	2 (18.2%)
Boraginaceae	*Plagiobothrys* spp.	10	0	1 (10%)	5 (50%)
Brassicaceae	*Brassica tournefortii*	17	1 (5.9%)	0	5 (29.4%)
Cactaceae	*Opuntia parryi*	19	0	0	3 (15.8%)
Cucurbitaceae	*Cucurbita foetidissima*	7	1 (14.3%)	0	1 (14.3%)
Cucurbitaceae	*Cucurbita palmata*	2	0	0	0
Euphorbiaceae	*Croton californicus*	5	0	0	3 (60%)
Euphorbiaceae	*Euphorbia* spp.	1	0	0	0
Fabaceae	*Acmispon glaber*	12	0	0	6 (50%)
Geraniaceae	*Erodium cicutarium*	1	0	0	0
Lamiaceae	*Salvia apiana*	1	0	0	0
Lamiaceae	*Salvia columbariae*	2	0	0	0
Lamiaceae	*Salvia mellifera*	7	0	1 (14.3%)	1 (14.3%)
Nyctaginaceae	*Mirabilis californica*	1	0	0	0
Onagraceae	*Camissonia* spp.	1	0	0	0
Papaveraceae	*Eschscholzia californica*	13	0	0	3 (23%)
Plantaginaceae	*Antirrhinum* spp.	1	0	0	0
Plantaginaceae	*Keckiella antirrhinoides*	3	0	0	2 (66.7%)

Polygonaceae	*Eriogonum fasciculatum*	215	9 (4.2%)	11 (5.1%)	16 (7.4%)
Solanaceae	*Solanum xanti*	3	0	1 (33.3%)	1 (33.3%)

Figures

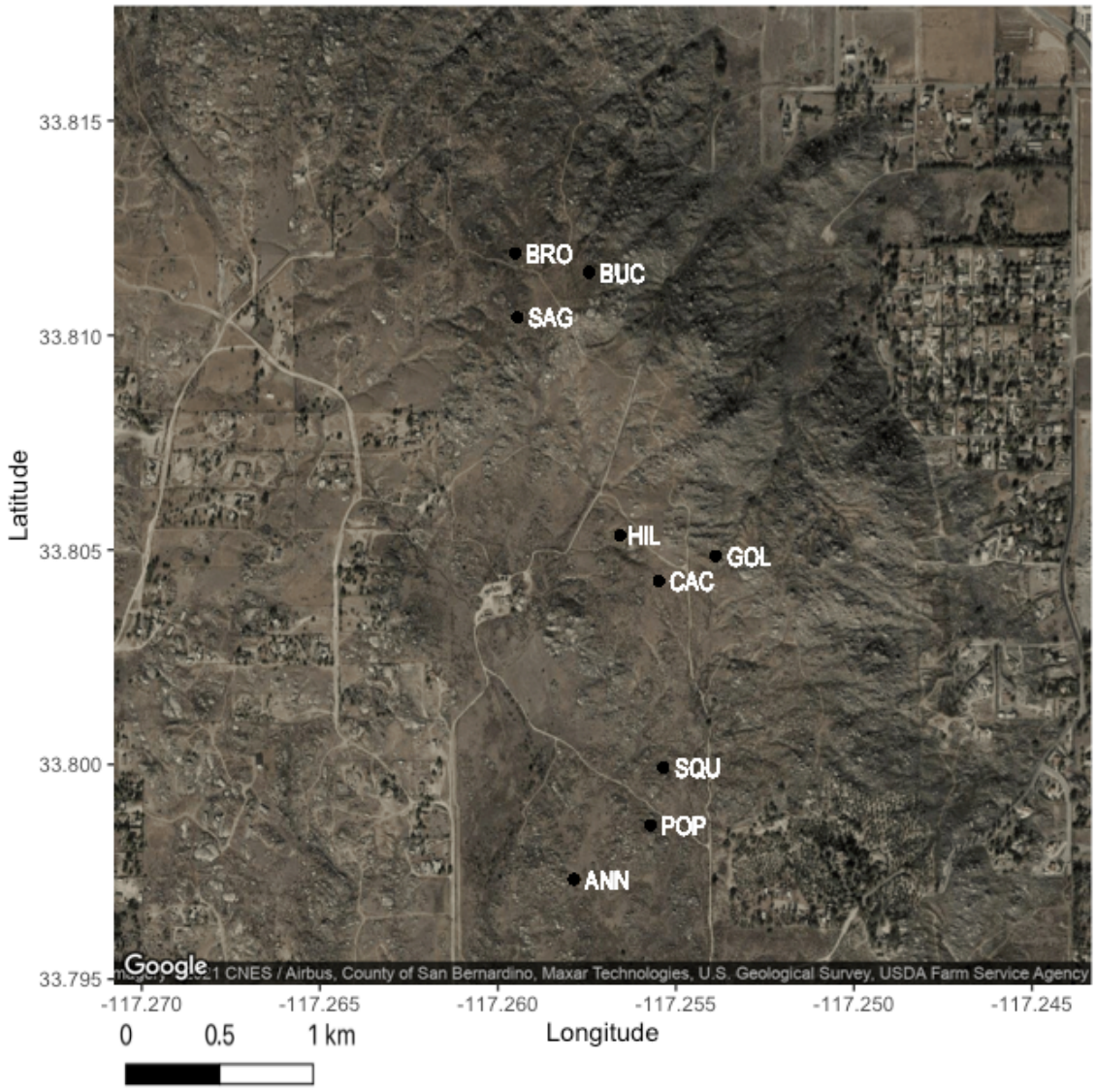

Figure 2.1: A map of sampling locations at Motte Rimrock Reserve (Perris, CA).

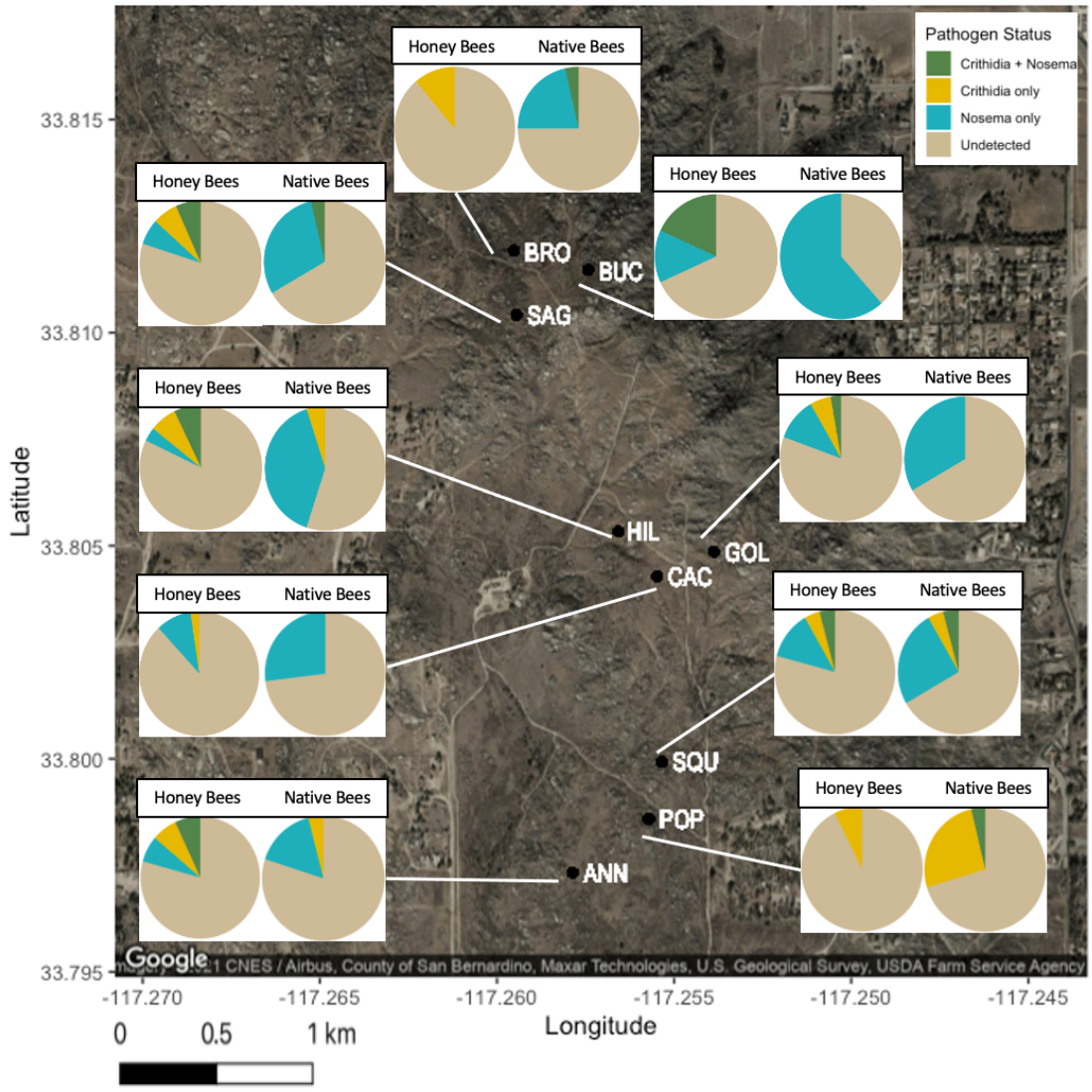

Figure 2.2: A map of sampling locations at Motte Rimrock Reserve (Perris, CA) in addition to the proportion of samples that screened positive for both *Crithidia* and *Nosema* (green), positive for *Crithidia* only (yellow), positive for *Nosema* only (blue), or screened negative for both *Crithidia* and *Nosema* ('Undetected') (beige).

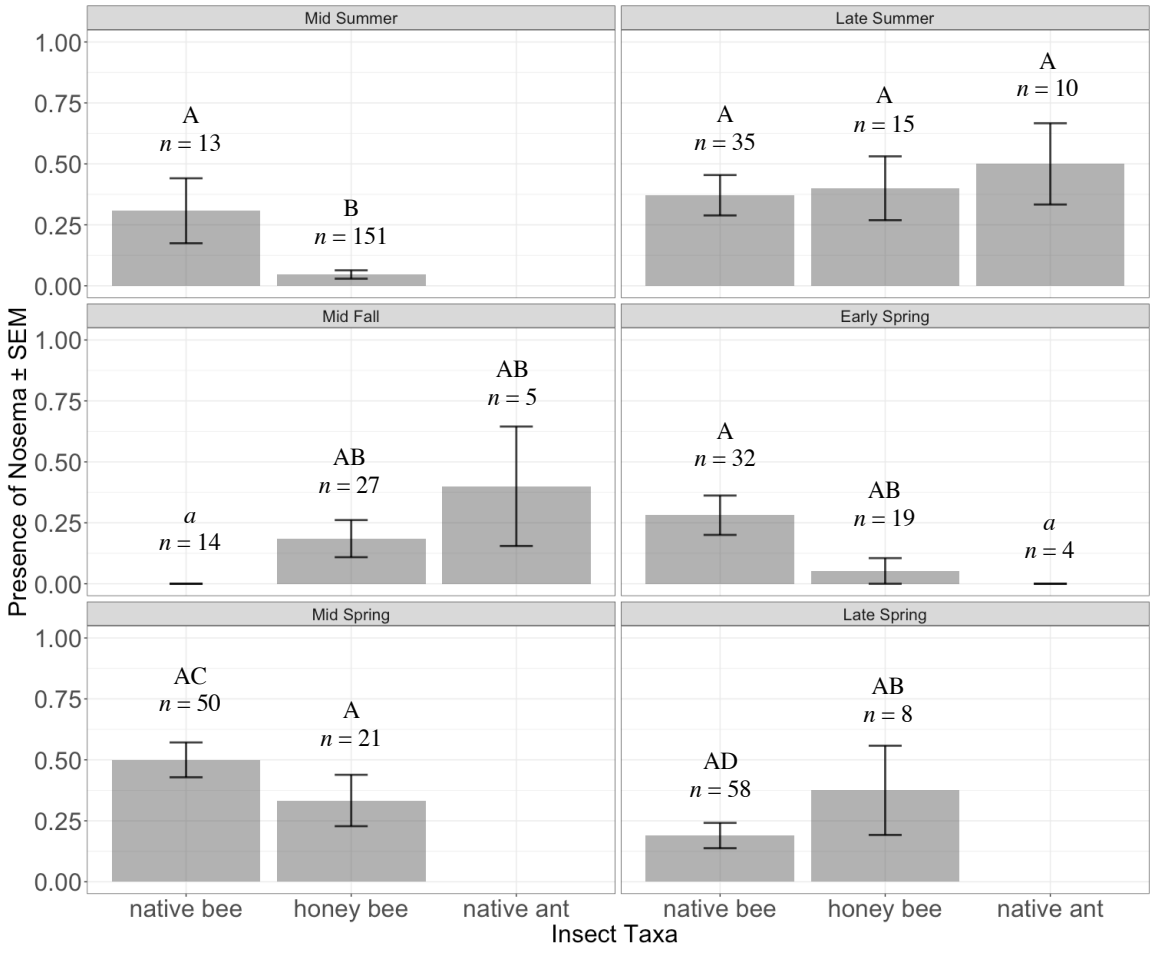

Figure 2.3: *Nosema* prevalence among insect taxa (native bees, honey bees, native ants) across sampling periods. Bars and whiskers represent the mean ± SEM. Any bar with the same uppercase letter as another bar indicates that those groups did not significantly differ. Bars without similar uppercase letters were significantly different. Columns with lower case italicized letters indicate that these groups have no variance (SE = 0) (‘*a*’) but could not be statistically supported as different during the *post hoc* analysis. The sample size indicates the number of pathogen screens conducted for a given insect taxa during a sampling period. Columns without bars and whiskers indicates that an insect taxon with a sample size of 0 during that sampling period. The order of panels from top left to bottom right indicates the sampling period order over the course of the study.

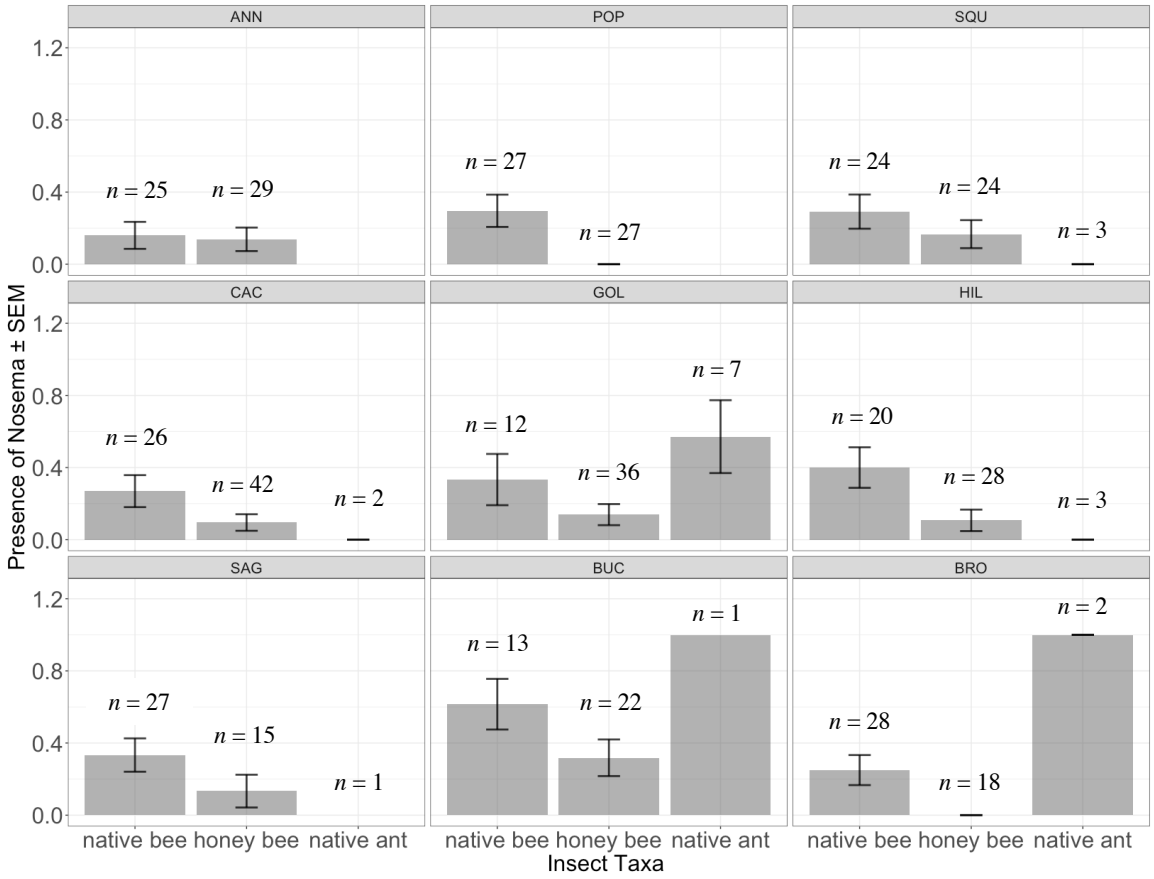

Figure 2.4: *Nosema* prevalence among insect taxa (native bees, honey bees, native ants) across sampling locations. Bars and whiskers represent the mean ± SEM. Letters are not included to indicate that the global analysis was significant, but the *post hoc* analysis did not reveal which groups were different. The sample size indicates the number of pathogen screens conducted for a given insect taxa collected from a particular sampling location. Columns without bars and whiskers indicates that an insect taxon with a sample size of 0 from that sampling location. The order of panels indicates sampling locations that were closer (e.g., top left and top middle) and further (e.g., top left and bottom right) at Motte Rimrock Reserve.

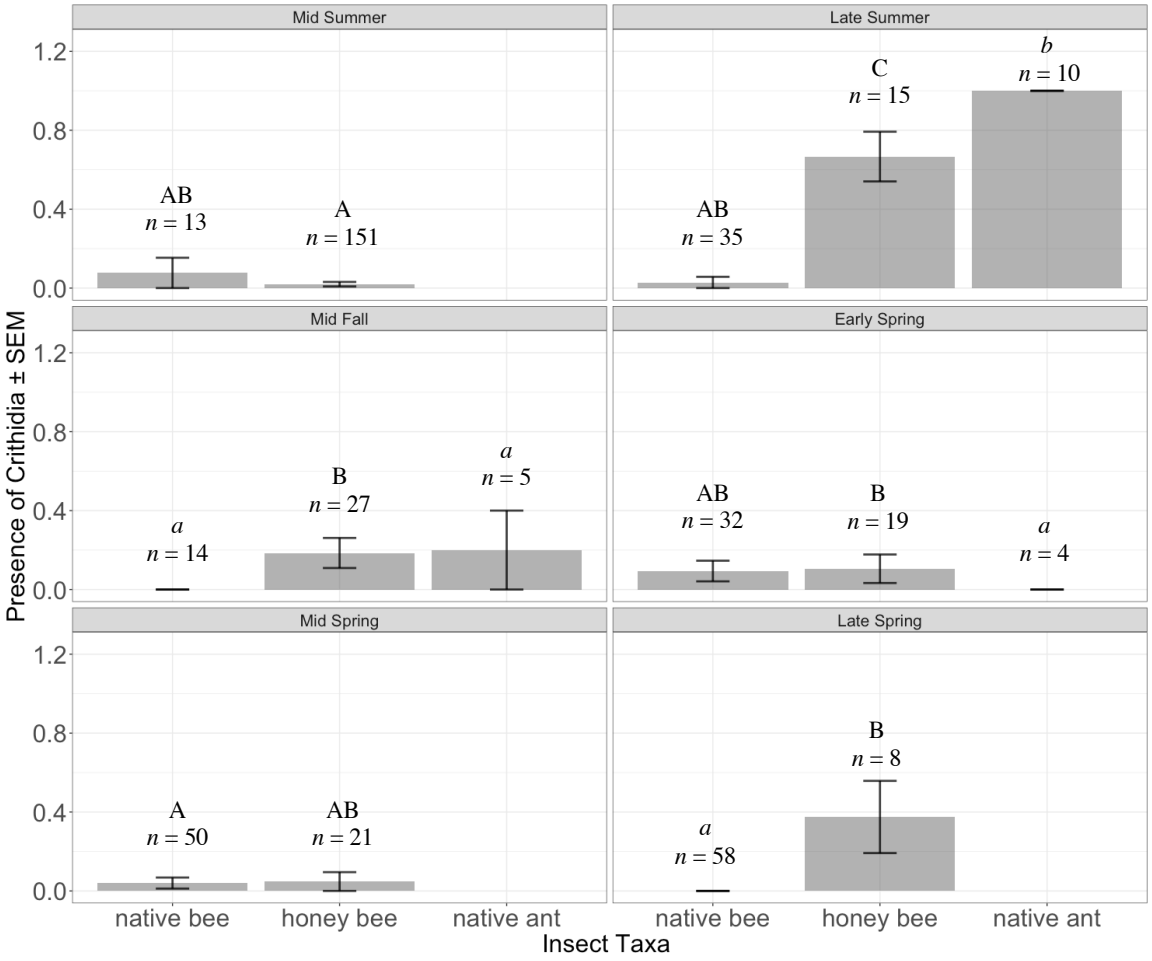

Figure 2.5: *Crithidia* prevalence among insect taxa (native bees, honey bees, native ants) across sampling periods. Bars and whiskers represent the mean ± SEM. Columns with lower case italicized letters indicate that these groups have no variance (SE = 0) and a mean equal to either 0 (‘*a*’) or 1 (‘*b*’) but could not be statistically supported as different during the *post hoc* analysis. The sample size indicates the number of pathogen screens conducted for a given insect taxa during a sampling period. Columns without bars and whiskers indicates that an insect taxon with a sample size of 0 during that sampling period. The order of panels from top left to bottom right indicates the sampling period order over the course of the study.

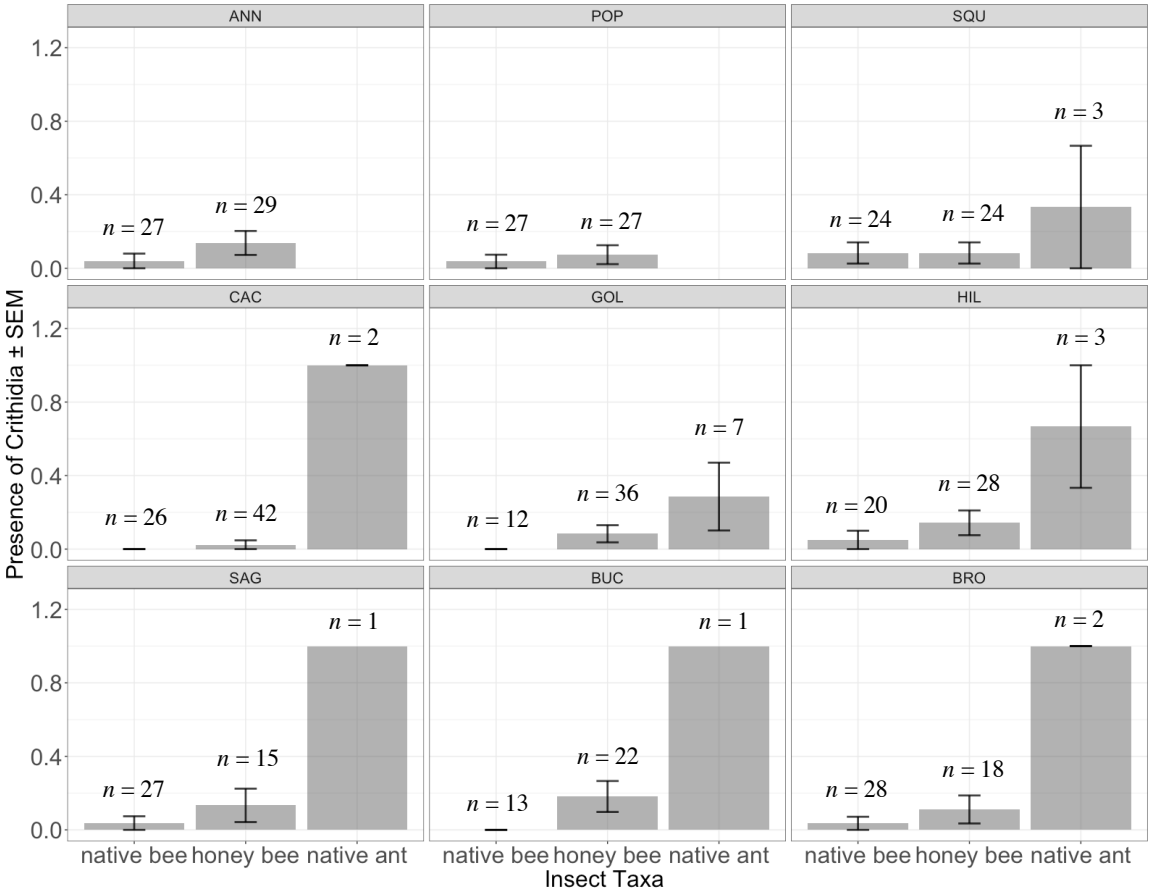

Figure 2.6: *Crithidia* prevalence among insect taxa (native bees, honey bees, native ants) across sampling locations. Bars and whiskers represent the mean ± SEM. Letters are not included to indicate that the global analysis was significant, but the *post hoc* analysis did not reveal which groups were different. The sample size indicates the number of pathogen screens conducted for a given insect taxa collected from a particular sampling location. Columns without bars and whiskers indicates that an insect taxon with a sample size of 0 from that sampling location. The order of panels indicates sampling locations that were closer (e.g., top left and top middle) and further (e.g., top left and bottom right) at Motte Rimrock Reserve.

Chapter 3: Conclusions

This thesis provides evidence that both *Nosema* and *Crithidia* are being detected in a broad diversity of wild bees and ants living in the studied coastal sage scrub habitat—a finding that merits further investigation. Of particular interest would be to determine whether pathogens are being transmitted between bees and ants. Since *Nosema* and *Crithidia* have not been previously detected among ants prior to this study, our experimental design in Chapter 2 was not designed to assess floral transmission explicitly.. Namely, ants visiting cookie baits does not directly represent species interactions occurring at flowers, thus preventing any direct conclusions about a mechanism of transmission between bees and ants currently. Now that these pathogens have been documented in ants for the first time, there are two potential transmission routes that future studies should address: (1) transmission between bees and ants at shared floral resources; and (2) transmission to ants through consumption of infected bees.

Shared flowers as a potential transmission mechanism between bees and ants

Ants commonly forage on flower nectar (Haber et al. 1981) and occasionally serve as important pollinators (de Vega et al. 2009). For floral transmission between bees and ants to occur, the question is not whether ants will visit flowers, but if ants and bees will come into sufficient direct and/or indirect contact with one another or with contaminated nectar in both space and time. Spatial resource partitioning is one potential mechanism that may limit pathogen transmission between ants and bees on flowers. For example, ants are attracted to extrafloral nectaries (Barônio & Del-Claro, 2018) and the

presence of these structures on flowers may limit interspecific interactions at flowers (Figure 3.1). Extrafloral nectaries are only present on a minority of flowering plants though (Marazzi et al. 2013) and their absence may force ants and bees to interact with the same floral structures. Future studies could examine the potential role of spatial resource partitioning at flowers as a mechanism that may mediate pathogen transmission between bees and ants by comparing a diversity of plant species in which extrafloral nectaries are either absent or present.

Ecological communities are not static entities, and we would expect resources to fluctuate across space and time. Temporal differences may also influence the transmission of pathogens between bees and ants (Figure 3.3). When floral resources are abundant, bees and ants may partition resources to facilitate coexistence. These conditions would likely favor intraspecific pathogen transmission if floral resource partitioning structures communities (Figure 3.3A). When resources are less abundant, bees and ants may have to compete for the same floral resources which would potentially increase the likelihood for these taxa to share flowers, potentially increasing the likelihood of transmission between taxa (Figure 3.3B).

During peak floral bloom, there should be a decreased probability for a host to encounter a pathogen (Figure 3.3A). However, when flowers become less abundant, there should be an increased probability for a specific host to encounter a pathogen (Figure 3.3B). Empirically, it has been shown in Pennsylvania agro-landscapes that bumble bees with less abundant spring floral resources were more likely to have higher pathogen loads (McNeil et al. 2020). While there is empirical support showing how floral abundance

influences pathogen prevalence, it is also important to think about this dynamic in terms of relative abundance since increases in floral resources should support more individuals in the environment, which may potentially yield a scenario in which concurrent changes in floral resources also dictates the number of individuals. Thus, the relative abundance of bees under peak and post-peak floral bloom conditions may lead to a more constant probability of pathogen encounter across time. Still, the differences in pathogen prevalence for communities at Motte Rimrock Reserve was not constant over time. If the probability of pathogen encounter was constant across time this would likely lead to consistent pathogen prevalence levels as well, and since we observed substantial variation in pathogen prevalence across time, this suggests that there are additional factors beyond the abundance of bees and flowers shaping observed pathogen patterns. Floral diversity may also play a key role in shaping pathogen prevalence. At Motte Rimrock Reserve, we have observed that floral resources are more diverse but less abundant across the landscape in the spring compared to the summer when floral resources are more abundant but less diverse (Allen & Rankin, *unpublished data*). Future studies should focus on quantifying fluctuations in relative floral resource abundance and diversity over time to see how this may influence pathogen patterns in coastal sage scrub communities.

Furthermore, scent cues may also structure interactions between bees and ants on flowers as both honey bees (Sidhu & Wilson Rankin, 2016; Unni et al. 2021) and bumble bees (Cembrowski et al. 2014) avoid ant harassment at flowers using scent cues. Therefore, floral interactions between ants and bees may be limited due to ant avoidance by bees. This avoidance behavior could also be a mechanism which limits the

transmission of pathogens between bees and ants (Figure 3.2). An experiment could test how the effects of longer floral visitation duration on ant-excluded flowers (Sidhu & Wilson Rankin, 2016) influence pathogen acquisition rates by bees, which could potentially elucidate a scent mediated transmission mechanism. Conversely, longer handling time on flowers or slower responses among infected bees (Koch et al. 2017) to leave flowers where ants are present could result in an increased likelihood of pathogen deposition via increased rate of defecation (*sensu* Figueroa et al. 2019). Thus, ants may influence foraging behaviors in which healthy bees may spend very little time or avoid flowers entirely when ants are present, but infected bees with impaired motor function may spend more time on these flowers—potentially increasing the likelihood of pathogen acquisition by ants.

Consumption of infected bees as a potential transmission mechanism to ants

Ants are voracious predators of arthropods (Cerdá & Dejean, 2011), including bees (Plentovich et al. 2021). However, there is a paucity of empirical evidence of prey to consumer pathogen spillover in wildlife (Malmberg et al. 2020), but such spillover events would have important ramifications for the consumer. In Hawaii, pathogen spillover via predation of honey bees is the suspected mechanism for observed patterns in *deformed wing virus* among invasive yellowjackets (Loope et al. 2019). As previously mentioned though, it is much more difficult to determine non-viral pathogen infections. Therefore, experiments would need to infect bees with *Nosema* or *Crithidia,* allow these pathogens to replicate to peak levels within the host (*sensu* Ngor et al. 2020), and then feed infected

prey items to *Nosema*/*Crithidia*-free ants to see whether pathogens could establish within an ant host after consumption of infected prey.

Infected prey may represent a poor-quality resource which could diminish consumer fecundity, survival, and longevity (Flick et al. 2016). Therefore, the ability for ants to distinguish between infected and healthy bees could lead to important fitness outcomes for the consumer. A future study could test for prey preferences among ants. This could be conducted in a buffet-style choice assay (*sensu* Wilson et al. 2010) where ants would be allowed to freely choose between healthy and infected bees (Figure 3.4). Additionally, ants could be forced to consume healthy and infected bees in a laboratory experiment to document whether fitness effects occur. Predation choices by ants may play out differently beyond the constraints of controlled experiments when considering how bees may be able to evade predation attacks. In fact, infected prey may be more likely to be captured by predators (Thomas et al. 2006). Ants may be more successful in capturing infected bees, especially when considering that bumble bees infected with *C. bombi* have reduced motor function and flower handling ability (Koch et al. 2017). Thus, while innate consumer preferences may occur in ants, the actual rates of consumption for infected or healthy bees may be different from these preferences and could have important consequences for ant population dynamics. It is not clear whether ants engage in predatory attacks on bees at flowers as there is a lack of empirical evidence in the literature for this interaction, although evasion behavior of ant predation by sweat bees at nest entrances has been documented (Wcislo & Schatz, 2003). A more realistic scenario

is one in which pathogen prevalence among ants via a consumption mechanism is primarily shaped by scavenging than predation.

Consequences of Nosema and Crithidia for bee and ant populations

Pathogens such as *Nosema* and *Crithidia* are clearly implicated in global bee declines (Goulson et al. 2015). While pathogen spillover from managed honey bees to both native, wild bees (Graystock et al. 2016) and ants (Sébastien et al. 2015) has been described previously, the results from Chapter 2 provide additional knowledge into a potentially intertwined pathosphere among honey bees, native bees, and ants. While it is still unclear whether *Nosema* and *Crithidia* are being shared among these insects, additional molecular approaches could help to resolve this ambiguity. If these pathogens are shared, then transmission could be limited or facilitated through mechanisms of shared floral resources or consumption of infected bees by ants, although such mechanisms have yet to be tested. These mechanisms, however, may not be mutually exclusive and likely have dynamic influences depending on environmental conditions. Such findings may define the potential for *Nosema* and *Crithidia* to not only accumulate within ant populations, but also to spillback into bee populations. The feedback loop between spillover and spillback would highlight novel pathogen transmission pathways among bees and ants.

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Figures

A) Extrafloral Nectaries Present

B) Extrafloral Nectaries Absent

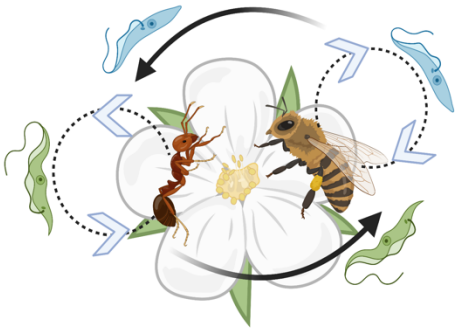

Figure 3.1: A visual depiction of how the presence or absence of extrafloral nectaries on flowers may potentially mediate pathogen transmission between bees and ants. A) Bees and ants may occupy different spaces on flowers when extrafloral nectaries are present which may support intraspecific pathogen transmission but may inhibit transmission between bees and ants. B) Bees and ants may occupy the same space on flowers when extrafloral nectaries are absent which may be conducive for both intraspecific pathogen transmission and transmission between bees and ants.

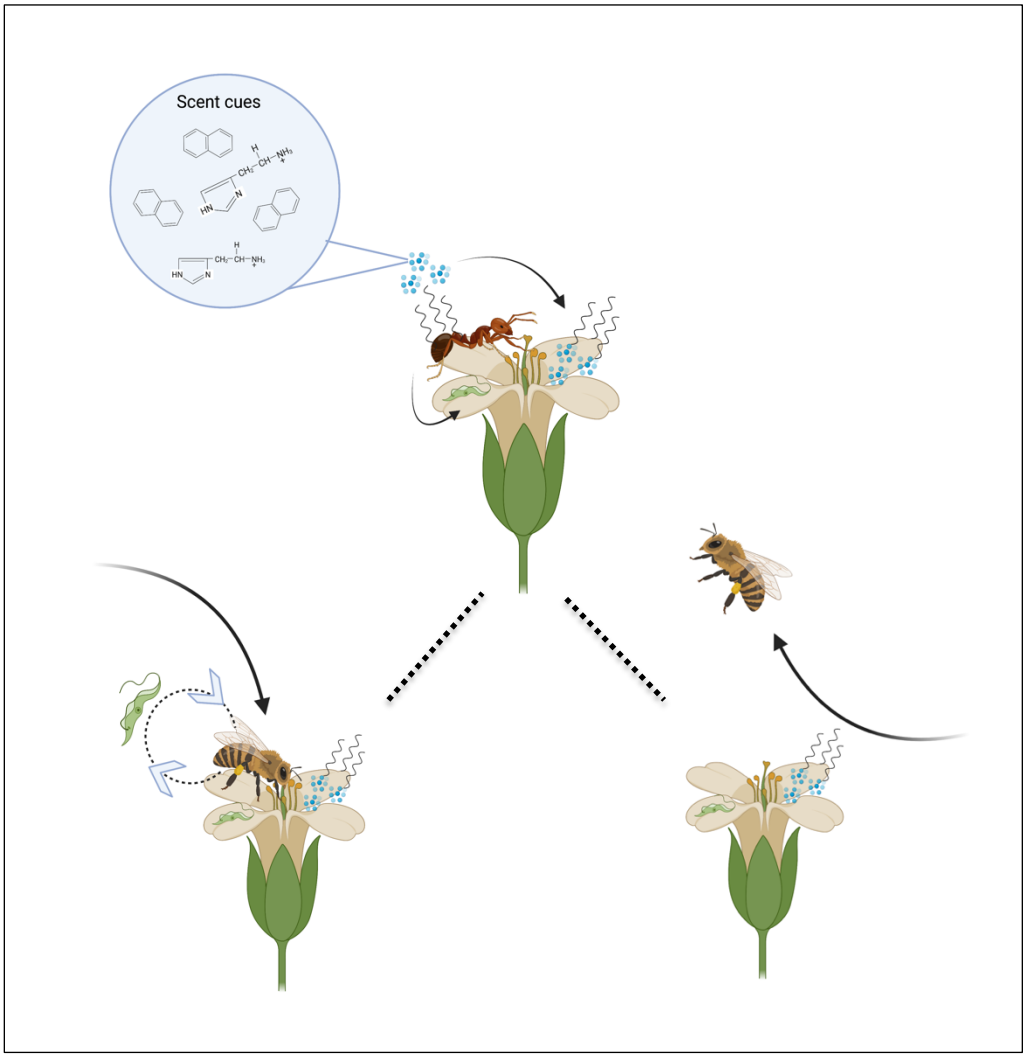

Figure 3.2: A visual depiction of how ant scent cues may mediate pathogen transmission between bees and ants. Ants may deposit scent cues and pathogens when visiting flowers (top). Bees may potentially detect these scent cues and avoid flowers when these cues are present and indirectly avoid encountering pathogens (bottom right). Bees that do not avoid flowers with scent cues present may potentially acquire pathogens left behind by ants (bottom left).

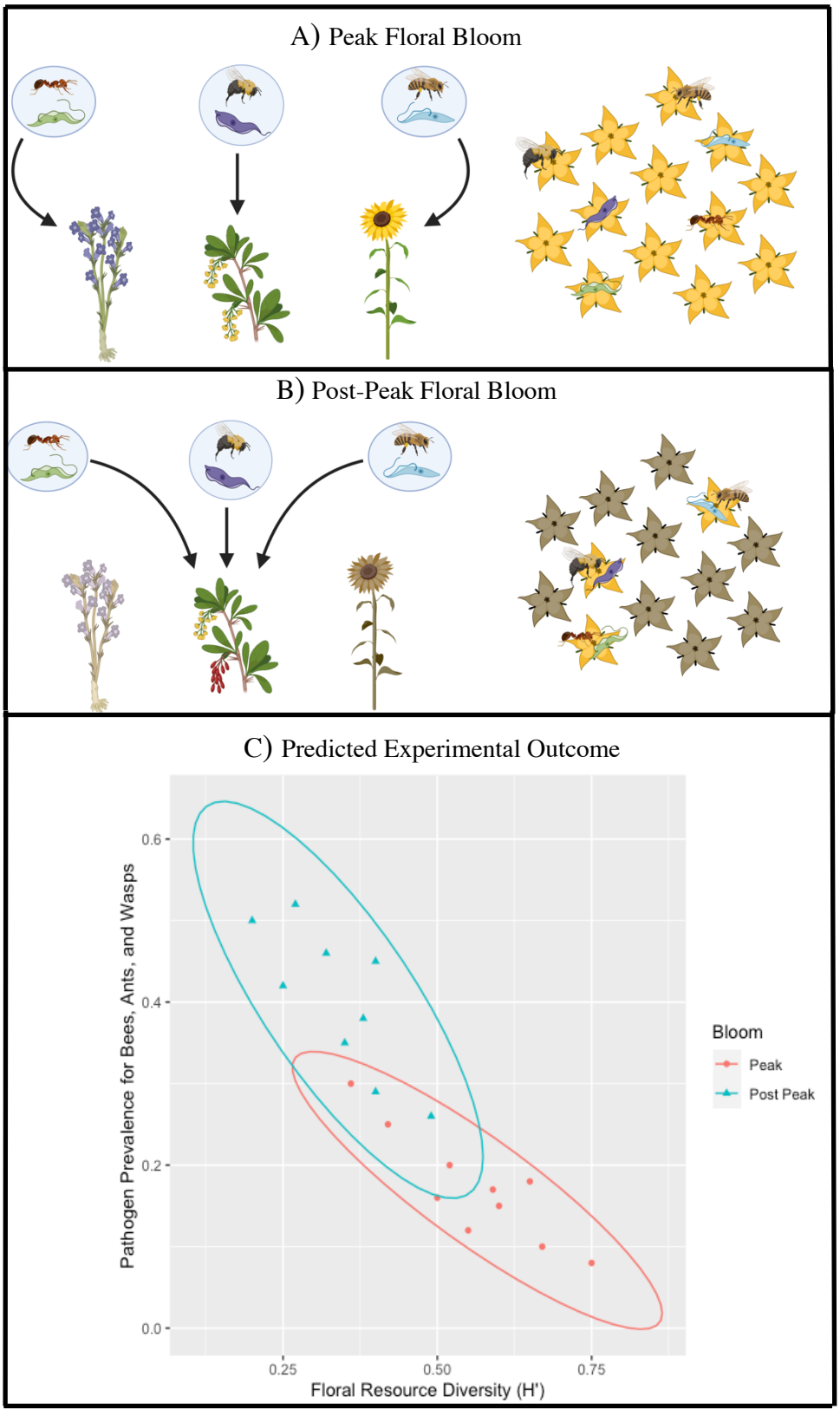
A) Peak Floral Bloom
B) Post-Peak Floral Bloom
C) Predicted Experimental Outcome
Pathogen Prevalence for Bees, Ants, and Wasps
0.0
0.2
0.4
0.6
0.25
0.50
0.75
Floral Resource Diversity (H')
Bloom
Peak
Post Peak

Figure 3.3: Pathogen prevalence among Hymenoptera may potentially be influenced by changes in floral resource abundance. A) During peak floral bloom, a diversity of blooming floral species would potentially allow insect taxa to partition resources, which may dilute the potential for interspecific transmission (left). Peak floral bloom for any plant species would also indicate a time period when floral resources are most abundant for that species. The higher abundance of resources to forage upon should decrease the probability of an insect encountering a pathogen on a flower (right). B) In post-peak floral bloom, the floral resource pool becomes limited as flowering species begin to senesce and bear fruit. Such conditions would potentially make it difficult for taxa to partition resources, which may amplify interspecific pathogen transmission (left). Resources will be less abundant during post-peak floral bloom, increasing likely competition for resources among floral visiting Hymenoptera, which may increase the probability of an insect encountering a pathogen (right). C) Floral resource diversity and abundance could be quantified for a community using Shannon's Diversity Index (H'). The prevalence of pathogens for a community, defined as the proportion of pathogen positive floral visiting Hymenoptera, could also be quantified and measured during peak and post-peak conditions. The predicted outcomes would include higher pathogen prevalence when floral resource diversity is low (blue ellipse) and lower pathogen prevalence when floral resource diversity is high (red ellipse). The partially overlapping ellipses indicates that pathogen prevalence during peak and post-peak floral bloom conditions would significantly differ based on centroid positions for these groups. Floral visitors are indicated by pictures of an ant, honey bee, and bumble bee (representing all native bees). Pathogens are indicated by green, purple, and blue trypanosome cells. Different flower colors indicate different flower species. Flowers in bloom have a brighter appearance in color. Flowers that are no longer in bloom are shaded or duller in color.

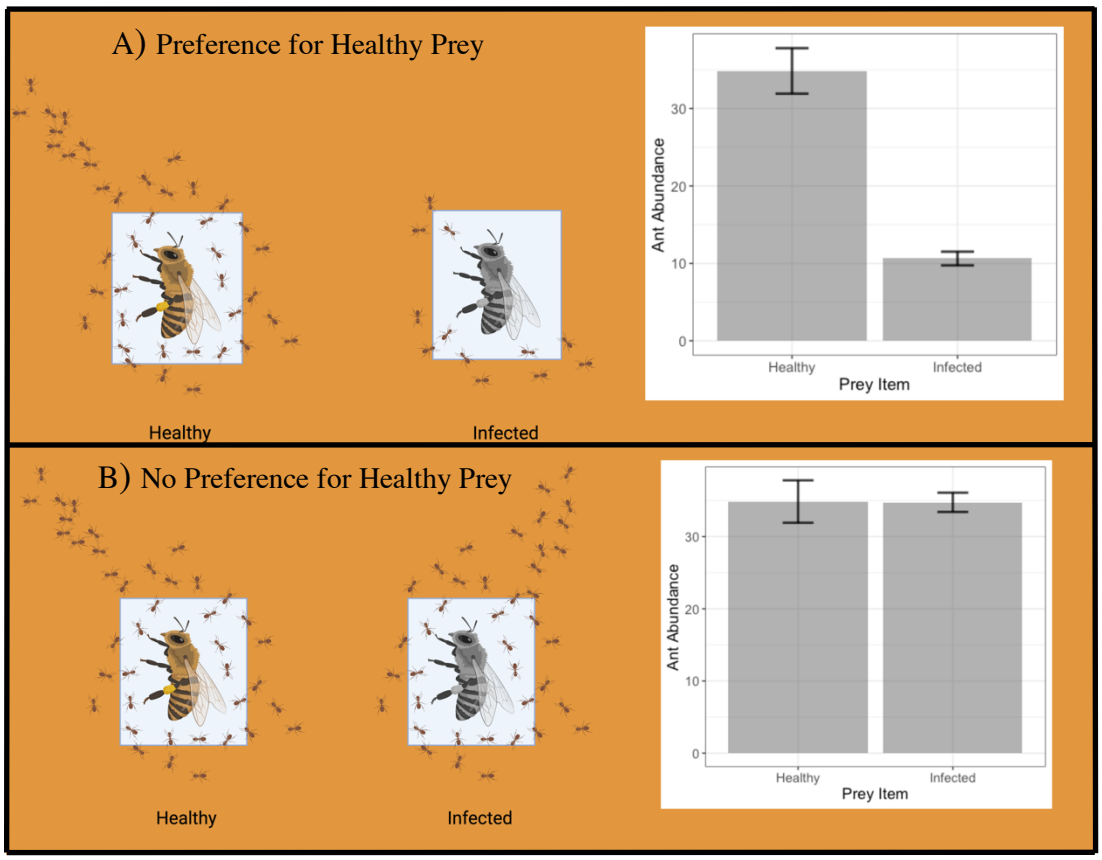

Figure 3.4: A visual depiction of how to study ant preferences for the consumption of healthy or infected bees using a buffet style predator choice assay (*sensu* Wilson & Holway, 2010). A) Ants show quantifiable differences between healthy (left) versus infected (middle) bees. The predicted outcome (right) would demonstrate that there are a higher abundance of ants actively foraging on the healthy prey items compared to infected prey items. B) If ants exhibit no discernible preference for healthy (left) versus infected (middle) bees, we would expect similar numbers of ants actively foraging on healthy bees as compared to on infected bees (right). The differences in these results would have different potential outcomes in the propensity of pathogen spillover events from bees to ants via a consumption mechanism.