IMPACTS OF INTER-COLONY DISTANCE, MITE HOST CHOICE, AND COLONY POLYANDRY ON THE HOST/PARASITE RELATIONSHIP BETWEEN *APIS MELLIFERA* AND *VARROA DESTRUCTOR*

by

MAXCY PEARLE NOLAN IV

(Under the Direction of Keith S. Delaplane)

ABSTRACT

The parasitic mite *Varroa destructor* Anderson and Trueman has become the single greatest threat to the honey bee (*Apis mellifera*) since its shift from its native host *Apis cerana*. This dissertation explores the impacts of inter-colony distance, mite host choice, and colony polyandry on the host/parasite relationship between these two organisms. Findings here showed that inter-colony distance affects Varroa mite counts within honey bee colonies, in that apiaries in which colonies were spaced 100 m apart contained lower mite population averages than 0 m or 10 m spacings. These findings suggest that mite populations are resource-regulated at a landscape scale. The link between reproductive value and risk assessment was examined in pre- and post-partum mites. Given that theory assigns greater reproductive value (predicted future offspring) to younger individuals, I hypothesized that a mite's reproductive value affects its propensity to disperse. In the study, both pre- and post-partum mites preferred newly emerged workers over pollen foragers; however, a bias for newly emerged bees was earlier and more strongly sustained among pre-partum mites. This suggests comparatively greater dispersal risk tolerance

among post-partum mites. The condition of polyandry, or multiple matings, among honey bees increases intra-colony genetic diversity and may play a role in nestmate discrimination. Additionally, recent evidence suggests a positive relationship between genetic variance and colony fitness. My results found no significant difference in drift or heterocolonial worker acceptance between colony classes of 5- and 15-drone inseminated queens, which are within natural mating numbers. Based on the results, nestmate recognition is not affected by intra-colony genetic variance encompassing natural levels. Furthermore, I measured metrics of colony strength, parasite resistance, comb building, and forager recruitment as evidence of the benefits of genetic variance to colony fitness. No significant differences were detected in any of the categories except in forager recruitment, which found an increase in forager recruitment in colonies with lower genetic diversity. These results suggest variation in colony genetic diversity encompassing natural mating numbers does not affect colony fitness.

INDEX WORDS: *Apis mellifera, Varroa destructor,* parasite transmission, host-parasite interactions, colony collapse, host choice, risk assessment, reproductive potential, polyandry, nestmate recognition, kin recognition, kin selection, genetic variance, social insect evolution

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DEDICATION

To my wife, daughters, parents, relatives, and friends, who love, encourage, inspire, and motivate me.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

My dissertation utilizes multiple experiments to further the understanding of *Varroa destructor* transmission among colonies of the honey bee, *Apis mellifera*. Varroa mites lack a free-living state and rely solely on their bee hosts for horizontal transmission between colonies (Sakofski and Koeniger 1988; Sakofski et al. 1990). My research examined three variables that affect horizontal transmission within and among colonies: inter-colony distance, mite choice, and colony polyandry.

Worker bees facilitate horizontal mite transmission through robbing and drifting behaviors (Greatti et al. 1992; Sakofski et al. 1990). Robbing occurs when the bees of one or more colonies attempt to enter another colony to steal honey (Free 1954b), whereas drifting occurs when a honey bee leaves one colony and joins another (Free 1958). Both behaviors are heavily influenced by inter-colony distance (Jay 1968). The average inter-colony distance observed in nature ranges from 304-4848 m (mean=2326 ± SD=1031; *n*=45; derived from Fig. 1, Seeley et al. 2015), whereas inter-colony distance in managed apiaries is far smaller, with distances < 1 m not uncommon. This environment supports drifting and robbing behaviors, facilitates greater contact between honey bees of different colonies, and ultimately raises horizontal parasite transmission and virulence (Bull 1994). Chapter 2 focuses on manipulating and replicating inter-colony distance to monitor the effects on Varroa mite population.

In addition to inter-colony distance, I studied effects of mite reproductive value (Chapter 3) on their risk tolerance and subsequent host choice. Theoretical models of life history predict that individuals of lower residual reproductive value will tolerate higher risk (Ghalambor and Martin 2001), enabling prediction of which individuals in a population will engage in risky dispersal behavior. I predict that pre-partum mites with higher reproduction potential will favor nurse bees who are behaviorally restricted to the nest, as hosts. Conversely, I predict that older mites who have already reproduced will be more likely to engage in risky dispersal behavior and express a higher rate of phoretic behavior on older forager bees who are more likely to drift into other colonies. These bees provide a greater opportunity for horizontal transmission; however, an increased mortality risk accompanies it. To study effects of mite reproductive value on risk tolerance and subsequent host choice, I used controlled lab conditions to observe the bee host choices of pre-partum and post-partum mites.

Chapter 4 examines the influence of queen mating number on honey bee worker drift. Honey bees, like other social insects, rely on nestmate recognition to maintain colony integrity (Downs and Ratnieks 1999; Hölldobler and Wilson 1990; Wilson 1971). The ability of guard bees to correctly identify nestmates from non-nestmates assists colonies in minimizing acceptance of drifted workers (Pfeiffer and Crailsheim 1998), presumably reducing horizontal transmission of mites at the same time. The decision to accept or reject is judged almost exclusively on odors unique to a colony (Breed et al. 2015; Buckle and Greenberg 1981; Downs and Ratnieks 1999; Page Jr et al. 1991), and a component of these odors, consisting of cuticular hydrocarbons, are at least partially controlled by genetics (Page Jr, et al. 1991). Because honey bee queens are polyandrous,

mating with an average of 12±6.3 drones each (Tarpy et al. 2004), numerous subfamilies of female workers reside within a single colony. As a result, bees have evolved secondary adaptions for nest-mate recognition (Breed et al. 1988). By utilizing instrumentally inseminated queens to control colony polyandry levels, I compared the nestmate recognition system of honey bee colonies in response to intra-colony genetic variation.

Honey Bee Evolution and Sociality

Honey bee study affords researchers the opportunity to peer into the complex and elaborate life of social organisms (Seeley 2014). Honey bees are by far the most intensively studied social insects, and as such are often used as a baseline for comparative studies on physiology and behavior (Wilson 1971). The honey bee is placed taxonomically with all other bees in the superfamily Apoidea. The apomorphy of both larvae and adults adapting a vegetarian lifestyle (Michener 1974) separates them from their sister group, the Sphecoid wasps. Within the superfamily Apoidea, eusociality has arisen at least eight times, utilizing parasocial and subsocial routes (Wilson 1971). The family Apidae, in particular, offers an excellent opportunity to study the evolution of social behavior, as its members exhibit different levels of sociality, allowing for the study of the evolutionary progression of sociality (Wilson 1971).

The honey bees (Tribe Apini, genus *Apis*) and stingless bees (Tribe Meliponini) constitute all members of the subfamily Apinae, of which all members are advanced or highly eusocial. This grade of sociality contains all the qualities of the (i) quasisocial (cooperative brood care), (ii) semisocial (reproductive castes), and (iii) eusocial (overlapping of generations) grades. Highly eusocial members additionally: (1) differ in complexity of social integration, (2) have female casts (workers and queens) of strikingly

different behavior, physiology, size, and external structure, and (3) possess gynes lacking structures necessary to collect and manipulate pollen, rendering them unable to survive outside their colonies. They (4) establish new colonies by swarming, and (5) colonies are long-lived. Members store food for adult and larval consumption in nests, but not in brood cells (6), and (7) rarely exhibit aggression among individuals of the same colony. (8) Larvae are fed, at least in a large part, with glandular secretions of workers (Wilson 1971).

It is generally accepted that the primitive state of the honey bee, and indeed all social insects, was that of a solitary individual that mated once (monandry) (Hölldobler and Wilson 2008; Hölldobler and Wilson 1990; Hughes et al. 2008; Wilson 1971). Kin selection theory provides the best explanation as to how evolutionary processes enabled these solitary ancestors to develop into colonies in which some members, namely sterile workers, relinquish reproduction in favor of helping their mother, the queen, reproduce (Bourke 2011a).

Kin Selection Theory

While social insects can vary in many ways, they all share one commonality: a reproductive division of labor (Queller and Strassmann 1998). The altruistic workers of social insects forgo their own reproduction in order to raise their brothers and sisters. This criterion is the basis of an entire branch of biology known as social biology that addresses the issue of how individuals can sacrifice themselves to the whole in this manner without being eliminated through natural selection. Darwin himself was perplexed by this issue when he first proposed his theory of evolution by natural selection (1860). How could one evolve to have no individual fitness?

The answer to this question was finally formalized and quantified by Hamilton (1964). The theory, known as Hamilton's inclusive fitness theory, or kin selection theory (Smith 1964), leads the study of social evolution and eusociality (Birch and Okasha 2015; Bourke 2011b; Gardner et al. 2011). Given that sterile workers enjoy no reproductive success and cannot be acted upon directly by natural selection, kin selection theory expands the unit of selection to the group or kin through genetic relatedness (Gardner et al. 2011). Rather than genes producing copies of themselves and increasing the direct fitness of the organism containing the genes (direct fitness), genes increase the fitness of relatives who share copies of them (indirect fitness) (Queller and Strassmann 1998), and the unit of selection shifts to the colony instead of the individual. It is under kin selection theory that we have a framework under which we can explore the evolution of social insects. One important subcomponent is the phenomenon of polyandry.

Polyandry

Polyandry, or multiple mating of a female with different males (Keller and Reeve 1994), is one of the central challenges to understanding the evolution of sociality in Hymenoptera (Mattila and Seeley 2007). Based on current evolutionary theory, monandry is a crucial precondition to eusociality in Hymenoptera, with polyandry evolving secondarily (Jaffé 2014). The extreme levels of altruism exhibited by workers of eusocial colonies are explained by kin selection theory under a monandrous mating scheme; they fit particularity well into eusocial Hymenoptera operating under a haplo-diploid reproductive system (Palmer and Oldroyd 2000) with a singly mated queen.

While high levels of relatedness played a critical role in the evolution of eusociality, only enforced altruism, also called coercion or policing, allows for the

extreme altruism seen in highly eusocial insect societies (Ratnieks and Helanterä 2009). Coercion reduces the incentive for workers to lay eggs, eliminating reproductive conflicts that were critical in the development of sociality (Ratnieks and Helanterä 2009; Ratnieks et al. 2006). In the primitive social nest, the mother, being larger and stronger, would have harassed her daughters into submission by physically overpowering them and eating any eggs that were laid. Once species began specializing into worker and reproductive forms, the mother evolved new forms of coercion, such as pheromones that suppress worker ovary development, while daughters began to police each other. This may be readily observed in honey bees today; any worker-laid eggs are quickly eaten by other workers. This level of policing is so efficient that only 0.10-0.1% of the workers in a honey bee colony attempt to reproduce (Ratnieks and Wenseleers 2008).

In some groups, workers lost totipotency, or the ability to reproduce, allowing queens of some groups to adapt multiple mating or polyandry (Ratnieks and Helanterä 2009). This is supported by Hughes et al. (2008) as they provide evidence that the ancestral state of eusociality is monogamy, and the evolution of polyandry occurs in lineages where workers have lost the ability to adapt a queen-like role as adults. A number of hypotheses have been brought forward to explain the existence of polyandry within social insects (Palmer and Oldroyd 2000), with the four most plausible being: (i) bet hedging against insufficient or deficient sperm, (ii) providing disease resistance via genetic diversity, (iii) reduction in the production of diploid males, (iv) creating a more stable and resilient system of division of labor (Oldroyd and Fewell 2007).

Nestmate Recognition

Polyandry also affects the nestmate recognition system of social insects (Ratnieks et al. 2011). The nests of honey bee colonies are constructed in cavities, usually with one small opening. This allows for guard bees, equipped with stings, to patrol the entrance and more easily defend the colony. Guards inspect incoming bees and decide whether to allow entry or to deny it, and thus defend the hive (Ratnieks et al. 2011). The recognition of conspecific intruders presents a challenge to guard bees, as intruders share many of the same nestmate recognition cues as the guard's colony (Ratnieks et al. 2011). To attain colony individuality, each colony possesses a unique blend of recognition odors (Breed 1983; Breed and Julian 1992; Breed et al. 1998; Breed et al. 1988; Page Jr et al. 1991) made up of, among others, cuticular hydrocarbons. These odors are known to be at least partially under genetic control, and therefore may be affected by queen polyandry (Page Jr et al. 1991).

Coevolution of the Genera *Apis* and *Varroa*

The genus *Varroa* (Acari: Varroidae) was, until recently, comprised of three species of specialized and obligate ectoparasites of social cavity-nesting bees (*Apis* spp.) found in Asia (Anderson and Trueman 2000). Members included *Varroa jacobsoni, V. underwoodi,* and *V. rindereri*, which were, and still are, restricted to Asia, Papua, New Guinea, and Indonesia. They parasitize a number of native honey bees, namely *A. cerana, A. nigrocincta,* and *A. koschevnikovi.* A fourth member of the genus was recognized in 2000, when Anderson and Trueman determined that mites previously identified as *V. jacobsoni* were instead a new species: *Varroa destructor*.

V. destructor had successfully host-shifted from *Apis cerana* to the western honey bee *A. mellifera*. Authors speculate that *V. destructor* were first exposed to western honey bees when the bees were imported to Asia via the trans-Siberian railroad around 1905 (Oldroyd 1999). After a period of time, mites were able to successfully reproduce and thrive on *A. mellifera* hosts and were transported back to Russia and then Europe. The first recorded case of *V. destructor* in the Americas was in 1971; the first case in the United States occurred in Wisconsin in 1987 (Oldroyd 1999).

There are nine described haplotypes of *V. destructor*, and only two of which are known to parasitize *A. mellifera* – the relatively avirulent Japan/Thailand haplotype and the more virulent Korean haplotype (Rosenkranz et al. 2010). It is the latter that is now globally distributed and considered the most detrimental honey bee parasite. As *V. destructor* is a relatively new parasite of the honey bee, the host/parasite relationship remains unbalanced, and the mite has proved highly detrimental its honey bee host.

The original host, *A. cerana*, has co-evolved with *V. destructor*. Both species possess behavioral and biological adaptations evolving from a long period of interspecific co-adaption (Rath 1999), and because of their co-adaptation, *A. cerana* are rarely harmed by the mites (Oldroyd 1999). A comprehensive review of the co-adaptions between *A. cerana* and *V. jacobsoni* (though the author is most likely describing *V. destructor*) can be found in Rath (1999). These adaptations include: (i) hygienic traits, (ii) population-dynamic aspects, and (iii) physiological aspects. Most notable is the fact that mite reproduction is almost exclusively restricted to drone brood in *A. cerana*. Colonies of *A. cerana* only produce drone brood periodically, and mites seeking to parasitize the brood are frequently detected and, subsequently, either removed or entombed (Rath 1999). This

particular bee behavior is not restricted to drone brood; it has been observed in worker brood as well. Grooming also plays a role in limiting mite populations (Delfinado-Baker et al. 1992).

Evidence exists to support the hypothesis that *A. mellifera* possesses traits that enable co-adaption to *V. destructor* infection (LeConte et al. 2007; Locke et al. 2012; Rinderer et al. 2001; Rinderer et al. 2010). While the exact mechanisms of resistance remain unclear, the ability of the bees to suppress mite reproduction and subsequent population explosion has been well documented (LeConte et al. 2007; Locke et al. 2012; Locke and Fries 2011). The discovery of wild honey bees infected with *V. destructor* that have survived for extended periods of time without human intervention suggests that the normal coevolutionary process, or "arms race," between host and parasite can occur; however, the use of acaricides by beekeepers has hindered the natural process in managed colonies by removing the selective disadvantage of being virulent (Locke et al. 2012). A stable host-parasite relationship is predicted to evolve in colonies living in the wild where natural selection tends to be the strongest (Seeley et al. 2015). Populations operating in a stable host-parasite relationship have been documented in the USA (Seeley et al. 2015), France (LeConte et al. 2007) and Sweden (Fries et al. 2006).

Varroa Mite and *Apis mellifera*

Susceptible *A. mellifera* colonies, once infested by Varroa mites, typically succumb to the damage dealt by the mites in one to three years (Fries et al. 2006; Korpela et al. 1992). Mites harm colonies in a number of ways.

Female mites lay eggs in honey bee brood, and developing mites feed exclusively on developing honey bees (Fries et al. 1994; Rosenkranz et al. 2010). Adults feed on the

hemolymph of both adult bees and developing larvae, reducing lifespan, decreasing body weight, and potentially spreading disease (DeJong et al. 1982; Dietemann et al. 2012; Rosenkranz et al. 2010).

Rosenkranz et al. (2010) reports 18 viruses that have been isolated from honey bees; many can be vectored by *V. destructor*. Besides merely transmitting them, mites increase the virulence of many honey bee viruses, most notability deformed wing virus (DWV) (de Miranda and Fries 2008; de Miranda and Genersch 2010). Without mites, DWV rarely manifests in overt infections, suggesting a long coevolutionary relationship in which both host and pathogen are well adapted. Martin et al. (2012) monitored DWV prevalence, colony viral titers, and DWV diversity among honey bee populations throughout the Hawaiian Islands as Varroa mites were first discovered there in 2007. In mite free areas, only 6-13% of the tested colonies were positive for DWV, compared to 75-100% in areas where Varroa mites were established. Furthermore, a millionfold increase in viral loads was detected in mite positive colonies compared to mite free colonies. The study also discovered a massive reduction in DWV diversity associated with Varroa mite infestation. The authors hypothesize that the presence of Varroa mites selects for particular variants of DWV, decreasing the diversity of strains that were present before mite introduction.

Virulence Evolution

Virulence is only adaptive to a parasite to the extent it promotes its own fitness (Fries and Camazine 2001). Early theory suggested that pathogens would evolve to become avirulent commensals, since any harm in the host would also harm the parasite (Bull and Lauring 2014). This theory is known as the “avirulence hypothesis” or “conventional

wisdom hypothesis," and was the leading hypothesis for virulence evolution until it was challenged in the mid-20th century. During this time, a better understanding of pathogen evolution revealed several ways in which virulence could be not only maintained, but selected for (Alizon et al. 2009; Ewald 1983; Levin 1996). A cornerstone of this new hypothesis argued that transmission and virulence are linked.

The linking of transmission and virulence suggests a trade-off whereby a parasite evolves higher transmission at the cost of increased virulence. If the benefit of greater transmission outweighs the cost of greater virulence, then virulence can be selected for. One example involves parasite reproduction: by increasing reproduction, a parasite can increase transmission, usually increasing virulence at the same time. Of significance is the concept that disease virulence evolution does not depend strictly on reproduction, but is multifaceted; contributing factors include vectored vs. directly transmitted pathogens, host longevity, novel hosts, pathogen replication rate, life span of pathogen propagule, population structure, and host density (Fries and Camazine 2001).

Horizontal vs. Vertical Transmission

Horizontal transmission is defined as parasite transmission between individuals of the same generation, while vertical transmission refers to parasite transmission between parents and offspring (Fries and Camazine 2001). Ecological theory generally maintains that horizontally-transmitted parasites select for an increase in virulence, and experimental studies such as Agnew and Koella (1997), Ebert (1994), and Ebert and Mangin (1997) support that hypothesis. Conversely, vertically-transmitted parasites select for a decrease in virulence (Anderson and May 1982; Lipsitch et al. 1995; Lipsitch et al. 1996). Pathogens that spread vertically must rely on successful reproduction of the host

for their own reproduction; therefore, any decrease in fitness that occurs in the host reduces the fitness of the parasite as well (Stewart et al. 2005). Virulence evolution theory predicts that vertically transmitted parasites will not persist in host populations without some degree of horizontal transmission for support (Lipsitch et al. 1995). The Varroa mite is transferred in honey bees both vertically, via infested adult bees leaving with a reproductive swarm, and horizontally through robbing and drifting behaviors.

Objectives of Research

The primary objective of my research was to further understanding of the horizontal transmission of the parasitic mite *Varroa destructor* among colonies of the honey bee *Apis mellifera*. I accomplished this in three ways: (1) investigating distance between honey bee colonies as a regulator of Varroa mite population at a landscape scale, (2) studying effects of a mite's reproductive value on tolerance for risky dispersal behavior, and (3) examining the influence of queen mating number on honey bee worker drift.

Together, these studies evaluate aspects of Varroa mite and honey bee biology and behavior as they pertain to the host/parasite relationship, with an emphasis on horizontal transmission within the context of a social insect system. Greater knowledge of the Varroa/honey bee relationship will facilitate the development of improved management techniques and practices, aiding the apiculture community in more effectively managing this major pest species.

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CHAPTER 2

DISTANCE BETWEEN HONEY BEE *APIS MELLIFERA* COLONIES REGULATES POPULATIONS OF *VARROA DESTRUCTOR* AT A LANDSCAPE SCALE[1]

[1] Nolan IV, Maxcy P. and K.S. Delaplane. 2016. Accepted by *Apidologie,* Reprinted here with permission of the publisher.

Abstract

Inter-colony distance of *Apis mellifera* significantly affects colony numbers of the parasitic mite *Varroa destructor*. We set up 15 apiaries, each consisting of two colonies. Each apiary pair was assigned an inter-colony distance of 0, 10, or 100 m. Colonies were rendered nearly mite-free, then one colony in each pair was seeded with 300 female mites (mite-donor colony), while the other remained uninoculated (mite-recipient colony). After four months of monitoring, a whole model analysis showed that apiaries in which colonies were spaced 100 m apart contained lower average mite numbers than 0 m or 10 m apiaries. There were interactions among colony type, distance, and sampling date; however, when there were significant differences mite numbers were always lower in 100 m apiaries than 10 m apiaries. These findings pose the possibility that Varroa mite populations are resource regulated at a landscape scale: near-neighbor colonies constitute reproductive resource for mites in the form of additional bee brood.

Introduction

Varroa destructor is the most damaging parasite of the European honey bee (*Apis mellifera* L.) in the world today (Rosenkranz et al. 2010). A critical regulation point of this and any host-parasite relationship is inter-host transmission which occurs either vertically from parents to offspring or horizontally between individuals within a population. In the context of a honey bee colony, we presume for our present purposes that evolution is acting primarily at the colony level (Wilson and Sober 1989; Queller and Strassmann 1998) which means that horizontal transmission is best understood as action occurring between colonies, not between individuals within a colony. Therefore,

horizontal transmission in the *A. mellifera* / *V. destructor* system occurs through adult bee drifting and robbing (Sakofski and Koeniger 1988; Sakofski et al. 1990).

Drifting results when a honey bee leaves one colony and joins another (Free 1958). This phenomenon is common in managed apiaries where honey bee colonies are often placed in rows and in close proximity to each another. In managed situations drifting is affected by hive arrangement, inter-colony distance, distance from windbreaks, presence of landmarks, direction of colony entrance, topography, and hive color (Jay 1965, 1966a, 1966b, 1968). Drifting is ultimately caused by homing errors made as foraging honey bees return to the colony (Free 1958); however, Forfert et al. (2015) showed that colonies with high mite infestation had significantly enhanced acceptance of drifters. They postulate the increase in drifter acceptance is attributed to an impaired ability for guard bees to assess incoming heterocolonial foragers. It has been shown in numerous studies that developing honey bees parasitized by mites are less involved in brood care, hive ventilation, food collecting (Annoscia et al. 2015), and reduced homing abilities (Kralj and Fuchs 2006). A model calculated by Pfeiffer and Crailsheim (1998) predicted that hives placed linearly 26 cm apart and facing the same direction contain up to 42 ± 6% alien workers.

High drifting rates lead to high mite transmission rates; reinfestation rates as high as 75.6 mites / colony / day have been recorded in initially mite-free colonies whose nearest-neighbor infested colonies were 200 m distant (Greatti et al. 1992). When Sakofski et al. (1990) monitored weekly immigration of mites throughout a season they found no difference in mite migration when colonies were placed within a row of infested nearest neighbors or when colonies were placed 10 m away from infested neighbors.

Frey and Rosenkranz (2014) found that colonies located in areas with high colony density (>300 colonies within flight range of test colonies) had significantly higher mite invasion over a 3.5-mo period compared to colonies in a low density area (50 small nucleus colonies treated for mites before the study). Immigration rate in high density colonies averaged 462 ± 74 mites per colony over the 3.5-mo period, while low density colonies received 126 ± 16 mites.

Mite reinfestation and subsequent population increase were attributed to an increase in honey bee colony density by Seeley and Smith (2015). Colonies in their study consisted of 24 hives painted the same color with entrances facing the same direction and placed ~1 m apart in high-density apiaries or 21-73 m apart in low-density apiaries. Colonies that swarmed in low density apiaries had lower mite numbers and were able to maintain low mite levels leading to an increase in winter survival. Colonies in high density apiaries showed a reduction in mites immediately after swarming; however, mite numbers quickly rebounded leading to increased winter mortality. This rebound in mite population in high-density colonies was attributed to an influx of mites via drifting and robbing from non-swarming colonies within the apiaries. The high-density apiary was found to have significantly more drone drift than the low-density apiary. This marked increase in drone drift is a potential explanation for the rapid transmission of mites among colonies in the high-density apiary.

Frey et al. (2011) found no significant difference in number of mites transferred from heavily infested colonies into colonies located at distances of 1, 30, 400, 1,300, or 1500 m. The number of invading mites per colony raged from 85 to 444 mites within the two-month test period. The authors noted that during the testing period there was little

forage available to colonies and therefore colonies at all distances potentially robbed the weakened and collapsed heavily infested colonies. This might explain the relatively equal number of transferred mites observed over varying distances.

Horizontal transmission of mites is known to occur through robbing and drifting even at great distances, and an increase in colony numbers within the flight range of any one colony amplifies the number of invading mites. Epidemiological theory predicts that a parasite's virulence evolves to higher levels in populations with higher levels of horizontal transfer of the parasite (Bull 1994; Nowak and May 1994). In the *V. destructor* / *A. mellifera* relationship, increasing mite populations, whether by horizontal transmission (immigration) or endemic growth, are associated with increasing host colony morbidity and eventual death (Harbo 1996, Delaplane and Hood 1999, Seeley and Smith 2015). Therefore, it is important to explore the effects of colony distance on horizontal transmission of Varroa mites not only because closer distances increase immigration which leads to greater populations of mites and greater colony morbidity, but also because increases in host population densities are predicted to select for more virulent strains of parasites.

Owing to a long history of beekeeping, there are two ways to think about inter-colony distance in the context of mite transmission and virulence in *A. mellifera*: that existing in natural unmanaged bee populations and that encountered in managed apiaries. Average inter-colony distances in nature range from 304-4848 m (mean=2326 ± SD=1031; *n*=45; derived from Fig. 1, Seeley et al. 2015), whereas distances in apiaries are smaller by orders of magnitude with inter-colony distances of 1 m not uncommon.

With a range of possibilities this wide, we decided to focus on and replicate inter-colony distance to nearest neighbor as a driver in mite emigration and population growth.

In the present study we placed mite-free colonies at distances of 0, 10, or 100 m from a nearest-neighbor mite-infested colony and monitored mite levels and subsequent colony strength over a season. Our design differs from others because it replicates inter-colony distance and standardizes nearest neighbor condition while approaching inter-colony distances realistic for both natural and managed situations.

Materials and Methods

The study utilized 15 apiaries, each comprised of two honey bee colonies. Each apiary pair was randomly assigned one of three inter-colony distances: 0, 10, or 100 m (5 apiaries each distance). Apiaries were located at least 3.2 km from each other or any other known honey bee colony and all within 24 km of Athens, Georgia, USA (33.9500°N, 83.3833°W). Hives within apiary were painted the same color and were faced in the same cardinal direction and elevation to normalize drifting propensity within apiary.

Colonies were started on 14-15 Jun 2012 from three-pound (1.4-kg) packages and mated queens purchased from the same supplier. Upon arrival all packages were rendered nearly mite-free by treatment with 2.8% oxalic acid treatment at the rate of 3.0 mL solution per 1000 bees using the protocol of Aliano and Ellis (2009) which is expected to reduce mite levels by >90%. Treatment was given three days after packages were made, and bees remained in packages for three days post-treatment. Packages were housed in standard 10-frame Langstroth hives with screen bottom boards. Each colony was given two drawn combs and eight undrawn waxed plastic frames. Honey supers were added

mid summer to accommodate incoming nectar. Queen excluders were used, and colonies were fed 1:1 sugar water mixture as needed.

One colony in each apiary pair was randomly selected to receive 300 mites (donor colony). Inoculations were carried out 31 Jul-9 Aug. Live mites were collected from off-site heavily infested colonies by dusting top bars with powdered sugar and collecting mites that fall through screen bottom boards onto a white piece of corrugated plastic. Mites from multiple colonies were collected in this fashion, pooled together in the field, brought back to the lab, and counted into 300-mite cohorts. Mites were gently washed under lukewarm water to remove sugar, transferred onto moistened filter paper, and kept in an incubator at 32° C and ~40% relative humidity until inoculation. All mite inoculations were performed the same day as mite collection and were carried out by removing a brood frame from the target colony, brushing off adult bees, laying the frame horizontally across the hive, and gently pouring 300 mites onto an area of open brood. The frame was left in this position until mites were able to enter brood cells or hold onto cells. The frame was then carefully returned to the colony.

Relative mite counts were made using 48-h sticky screen counts on bottom boards on 14-19 Jun, 19-22 Jun, 29 Jun-2 Jul, 13-17 Jul, 27-29 Aug, 10-12 Sep, 25-27 Sep, 8-10 Oct, 24-26 Oct, and 13-15 Nov. Only sampling episodes from 27-29 Aug through 13-15 Nov were included in statistical analyses since donor colonies were not inoculated until 31 Jul-9 Aug; however, sampling prior to donor inoculation was done to ensure that colonies were as free of mites as possible. Baseline mean mites collected over all colonies for the first sampling episode, 14-19 Jun, was 167.6 ± 31.2 (mean ± SE). The means had reduced to 4.1 ± 1.3 by the subsequent episode, 19-22 Jun. A treatment using

the miticide Amitraz was administered after the 19-22 Jun sampling episode to further lower incipient mite levels. On the subsequent two sampling episodes, 29 Jun-2 Jul and 13-17 Jul, mean mite numbers had dropped to 1.4 ± 0.4 and 0.1 ± 0.1, respectively. On the 13-17 Jul sampling episode no colony had more than one mite on a 48-h sticky screen. In addition to relative sticky screen counts, total mite populations were determined at the start and conclusion of the study. Obtaining total mite populations required determining number of mites in brood and summing with phoretic mites on adult bees. Mites in brood was estimated by uncapping 100 worker bee brood cells and inspecting for mites. Phoretic mites were assessed using the alcohol wash method (~300 adult bees) (Dietemann et al. (2012).

Total adult bee population, capped worker brood, and capped honey were estimated following section 4.2 in Delaplane et al. (2013). By knowing total adult bees and total capped brood we were able to estimate colony mite populations. The ratio of mites in brood to total mites in each colony was determined as a proxy measure of fecundity of the mite population as described by Harbo (1996).

Analyses of 48-h sticky screen counts were conducted using the mixed model GLIMMIX procedure (SAS Institute 1992) recognizing inter-colony distance (0, 10, or 100 m), colony type (mite-donor or -recipient), and sampling episode as fixed effects and apiary replication as random effect. The data were analyzed using a GLIMMIX model coded for a Poisson distribution to account for conditional residuals showing skewness in the data. Tests were run for all two- and three-way interactions among fixed effects. Model means are reported for distance, colony type, and relevant interactions, but multiple comparisons (using Holm-Tukey) were run on least squares means.

The colony strength analysis also used the mixed model GLIMMIX procedure (SAS Institute 1992) recognizing inter-colony distance and colony type as fixed effects. In the case of bee population and capped brood cells the initial values for these parameters at start-up were included as covariates but later discarded when they failed to explain any variation in the models.

Results

48-h sticky screen mite drop counts were significantly affected by all three main effects: apiary inter-colony distance (F=3.8; df=2,12; P=0.05), colony type (mite-donor or -recipient) (F=17.6; df=1,12; P=0.001), and sampling episode (F=54.0; df=5,120; P<0.0001; Table 2.1). Additionally, there were significant interactions between colony type*sampling episode (F=8.2; df=5,120; P<0.0001; Fig. 2.1), distance*sampling episode (F=3.6; df=10,120; P=0.0003; Fig. 2.2), and colony type*distance*sampling episode (F=2.1; df=10,120; P=0.03; Fig. 2.3).

When pooled by apiary inter-colony distance, the whole model analysis showed that mean 48-h mite counts were separated by Holm Tukey in the following pattern: 100 m apiaries < (0 or 10 m apiaries) with means of 6.0 ± 0.9 (mean ± SE), n=60 in 100 m apiaries; 9.4 ± 1.0, n=60 in 10 m apiaries; and 9.2 ± 1.3 n=60 in 0 m apiaries.

When pooled by colony type, mite-donor colonies had significantly more mites 11.4 ± 1.0 n=90 than recipient colonies 5.0 ± 0.6 n=90.

Table 2.1 shows model means for 48-h screen counts pooled by sampling episode. The data show that mite populations significantly increased over the study period and then moved downward on the last sampling episode, a pattern typical of mite populations as winter approaches and honey bee brood production contracts.

Figure 2.1 shows the interaction of colony type and sampling episode. Donor colonies had significantly higher sticky screen counts than recipient colonies on all episodes except 24-26 Oct.

Figure 2.2 shows the interaction between apiary inter-colony distance and sampling episode. Mean mite counts on 48-h sticky screens were significantly higher in 10 m apiaries compared to 100 m apiaries on both the 25-27 Sep and 13-15 Nov sampling episodes. Otherwise there were no differences by distance on other episodes, nor did the patterns necessarily match 25-27 Sep or 13-15 Nov.

Figure 2.3 shows interactions among colony type*distance*sampling episode. For donor colonies, mean mite counts were significantly higher in the 10 m apiaries compared to 100 m apiaries on the 25-27 Sep and 13-15 Nov sampling episodes. For recipient colonies, mean mite counts were significantly higher in 10 m apiaries compared to 100 m apiaries on the 25-27 Sep and 8-10 Oct sampling episodes. There were no differences among distances by colony type on other sampling episodes, nor did the patterns necessarily match those episodes in which differences occurred.

Analyses of ending strength parameters found no significant effects of distance and colony type on adult bee populations, capped brood cells, total mites per colony, nor percent mites in brood ($P \geq 0.05$). Nevertheless, natural means and *n* for each strength parameter are provided in Table 2.2, grouped by apiary inter-colony distance.

Discussion

Our results add to a growing base of evidence that spatial structure of honey bee communities, in particular inter-colony distance, significantly affects colony Varroa mite numbers. By varying and replicating inter-colony distance over multiple apiaries we were

able to detect effects of nearest-neighbor mite-source colony on mite transmission to uninfected colonies. Relative mite numbers (48-h mite count) were measured over a 4-month period in order to observe and compare changes in mite levels at different distances over time.

Mite numbers increased steadily from the 27-29 Aug through 24-26 Oct sampling episodes and then showed a significant decrease in the 13-15 Nov episode (Table 2.1). This result is a predictable outcome of the fact that mite population growth is regulated in part by seasonal availability of bee brood (Fries et al. 1994; Calis et al. 1999; Vetharaniam 2012).

The trend for mite increase was similar in both donor and recipient colonies, with recipient colonies being significantly and predictably lower in all but one sampling episode (Fig. 2.1). When pooled, donor colonies had significantly more mites than recipient colonies (see Results).

We cannot discriminate whether mite growth over time was caused by drift, endemic mite reproduction or a combination of the two, but finding overall mite levels significantly lower in the 100 m apiaries compared to 10 m apiaries suggests that drifting plays a significant part. This interpretation is supported by previous studies showing that increases in horizontal transmission directly correlate to increases in drift brought about by higher colony densities and smaller inter-colony distances (Sakofski and Koeniger 1988; Sakofski et al. 1990; Sakofski 1991; Frey et al. 2011; Seeley and Smith 2015). On the interaction analysis (Fig. 2.2) apiaries grouped by sampling episode differed depending on distance. However, on dates where significant differences were observed, the pattern was always 100 m apiaries having fewer mites than 10 m apiaries, with 0 m

apiaries intermediate. It appears that the 0 m and 10 m colony distances were biologically indistinguishable with regard to bee drift and mite transmission, and we cannot offer a biological speculation why the 0 m apiaries interacted as intermediates.

The consistency of 100 m apiaries having fewer mites than 10 m apiaries is sustained in the three-way interaction of distance*colony donor type*sampling episode (Fig. 2.3). The continuity of lower mite numbers in the 100 m apiaries suggests a biologically meaningful threshold. This finding is consistent with that for the comparatively large inter-colony distances found in nature (range=304-4848 m; see Introduction, Seeley et al. 2015).

We expected effects on mite numbers across recipient colonies at different inter-colony distances due to varying rates of horizontal mite transmission; however, variation across donor colonies was unexpected. Furthermore, donor colonies followed similar mite progression patterns observed for recipient colonies (Fig. 2.3). One hypothesis that explains higher mite numbers in donor colonies in more closely-spaced apiaries posits that competition for larval hosts is less keen in those apiaries than in apiaries with colonies 100 m from their nearest neighbor. An increase in horizontal transmission enabled mites to more quickly exploit brood of the nearby and relatively mite-free recipient colonies. Indeed, an important component regulating colony mite population growth is availability of honey bee brood (Calis et al. 1999). Our findings suggest the possibility that this intra-colony mite population model can be expanded to the level of colony community at a landscape scale.

As shown in Table 2.2 we found no effects of apiary inter-colony distance on numerous proxy measures of colony fitness. This result is likely an artifact of the

relatively short time scale of the study; honey bee colonies with low initial mite populations do not show deleterious effects of mite infestation in temperate latitudes such as Georgia, USA until at least two seasons of unregulated growth (Calis et al. 1999). By utilizing colonies that were virtually mite-free at the onset, even donor colonies seeded with 300 adult female mites failed to reach the economic treatment threshold of 59-187 mites per 24-h sticky screen drop established by Delaplane and Hood (1999) for the American Southeast and later confirmed by Delaplane et al. (2010).

However, the close association between mite population growth and increasing colony morbidity is firmly established (Harbo 1996; Delaplane and Hood 1999; Delaplane et al. 2010), and the decrease in apiary-level counts of parasitic Varroa mites we detected at increasing inter-colony distances is consistent with edipidemiological theory that predicts decrease in parasite transmission and virulence at decreasing host densities (Bull 1994; Nowak and May 1994; Lipsitch et al. 1996; Fries and Camazine 2001; Schmid-Hempel 2011). Furthermore, crowding of colonies within apiaries (Seeley and Smith 2015) and crowding of apiaries within landscapes (Frey and Rosenkranz 2014) have been independently shown to increase mite transmission. The current study builds upon these and other studies by replicating inter-colony distance and detecting evidence of Varroa mite population regulation by brood availability at the level of landscape.

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Table 2.1 Model means (± SE) for 48-h mite sticky screen drop counts pooled by sampling episode over all distances and mite-donor/-recipient colonies. Counts with different letters are significantly different at $P<0.001$. Analyses were run on least squares means. In all cases, $n=30$.

27-29 Aug	10-12 Sep	25-27 Sep	8-10 Oct	24-26 Oct	13-15 Nov
2.6 ± 0.4a	3.8 ± 0.5b	4.7 ± 0.6b	6.1 ± 0.7c	12.9 ± 1.3d	7.5 ± 0.8c

Table 2.2 Natural means (± SE) for adult bee populations, capped brood cells, total mites per colony, and percent mites in brood. In all cases $n=10$.

	0 m apiaries	**10 m apiaries**	**100 m apiaries**
Adult Bees	7504 ± 802	7956 ± 719	8855 ± 1139
Capped Brood	669 ± 479	786 ± 307	1288 ± 557
Total Mite Population	319 ± 63	589 ± 114	453 ± 118
Percent Mites in Brood	7% ± 5	10% ± 5	14% ± 7

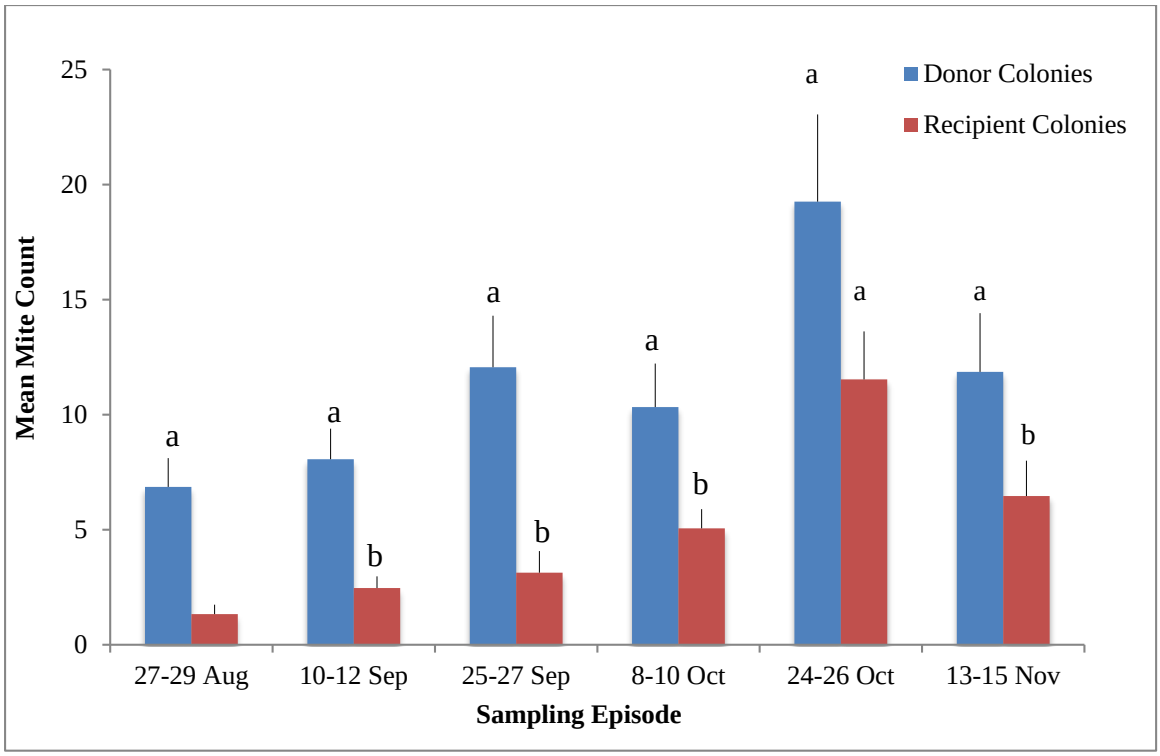

Figure 2.1 Interaction between colony donor type (mite-donor or -recipient) and sampling episode. Episodes before donor colonies were inoculated with mites are omitted. Different letters indicate significant differences between colony type within sampling episode. Error bars represent SE of the least squares means separation.

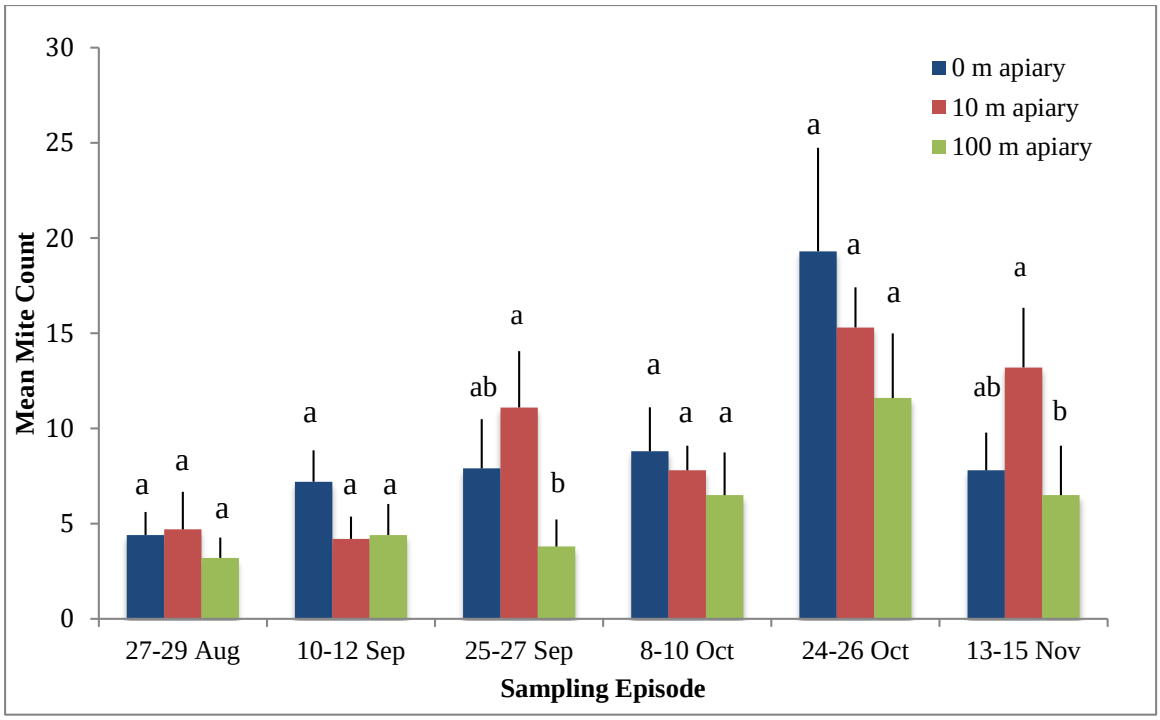

Figure 2.2 Interaction between apiary inter-colony distance and sampling episode. Episodes before donor colonies were inoculated with mites are omitted. Different letters indicate significant differences among colony distances within sampling episode. Error bars represent SE of the least squares means separation.

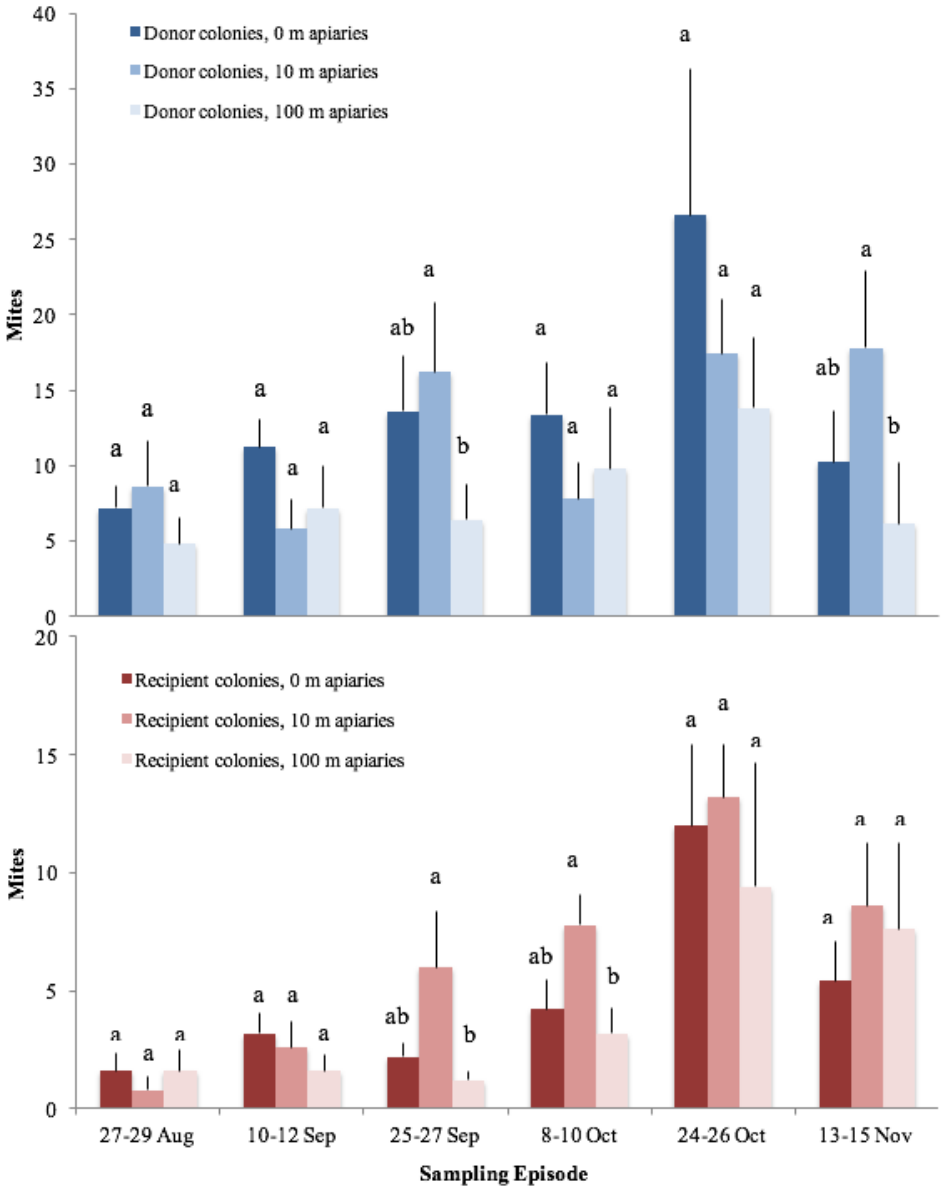

Figure 2.3 Interaction between colony distance, colony donor type (mite-donor or -recipient), and sampling episode. Episodes before donor colonies were inoculated with mites are omitted. Different letters indicate significant differences among colony distances within colony donor type (mite-donor or -recipient) and sampling episode. Error bars represent SE of the least squares means separation.

CHAPTER 3

DISPERSAL RISK TOLERANCE IS MEDIATED BY REPRODUCTIVE VALUE IN THE MITE *VARROA DESTRUCTOR*[1]

[1] Nolan IV, Maxcy P. and K.S. Delaplane. To be submitted to *Insectes Sociaux*

Abstract

The ecto-parasitic mite *Varroa destructor* is the greatest threat to modern apiculture. Mite reproduction happens exclusively inside cells of bee brood, and phoretic attachment to forager bees is the mite's mode of emigrating to new colonies and accessing new bee brood. Given that theory assigns greater reproductive value (predicted future offspring) to younger individuals, we hypothesize that a mite's reproductive value will affect its propensity to disperse. In lab assays, post-partum mites and pre-partum mites were offered a choice of newly emerged homocolonial worker bees, homocolonial pollen forager bees, or heterocolonial pollen foragers. Both pre- and post-partum mites preferred newly emerged workers over either pollen forager type; however, a bias for newly emerged bees was earlier and more strongly sustained among pre-partum mites. This suggests comparatively greater dispersal risk tolerance among post-partum mites that have already successfully reproduced.

Introduction

Successful parasites must not only exploit a host efficiently, but also assess host conditions to abandon overexploited hosts in favor of new more profitable ones (Cervo et al. 2014). The choice between staying at a proven resource or dispersing to a new one is a high stakes decision for all parasites. The study of dispersal incorporates aspects of ecology, genetics, behavior, and evolution. Indeed, one can argue that the field of epidemiology is largely the study of dispersal biology applied to pathogens (Lidicker Jr and Stenseth 1992). Applying theoretical models of life history stating that individuals of lower residual reproductive value will tolerate higher risk (Ghalambor and Martin 2001) allows one to make behavioral predictions about which individuals within a population

will disperse, with reproductive value being defined as the mean amount of future reproductive success based on the individual's age and sex (Williams 1966).

Here, we investigate the effect of reproductive value on dispersal risk tolerance in the parasitic mite *Varroa destructor*. This was accomplished under controlled lab conditions by comparing high- and low-risk host choices in pre- and post-partum Varroa mites. We predict that because a majority of female Varroa mites complete fewer than two reproductive cycles (DeRuijter and Calis 1988; Fries et al. 1994), pre-partum mites have a higher reproductive value and will exhibit lower risk tolerance by preferentially parasitizing low-risk hosts – in our case, young homocolonial bees. In contrast, post-partum mites will exhibit higher risk tolerance and parasitize older forager bees, either homocolonial or heterocolonial (ie. robbers), at a comparatively higher rate.

A dispersing mite subpopulation was detected by Kuenen and Calderone (1997) who, using mites of unknown age, showed that mites chose high-risk forager bees 20% of the time. Dispersal behavior in post-partum mites is driven by shrinking brood resources in severely-parasitized host colonies (Boecking and Genersch 2008), increasing reproductive competition for fewer brood cells (Cote et al. 2007; Kubisch et al. 2013), and the rewards of exploiting new hosts (Cote and Clobert 2010). We hypothesize that a mite's reproductive value will affect its propensity to disperse: all mites on average will show a preference bias for homocolonial bees because these bees offer the lowest risk opportunity for finding open brood and reproducing. However, post-partum mites will show a comparatively greater propensity to parasitize high-risk forager bees, affording these risk-takers an opportunity to emigrate to new resources at a landscape scale.

Materials and Methods

Collecting and marking mites

Mites were collected from infested colonies maintained by the University of Georgia Honey Bee Lab (33.9500° N, 83.3833° W) with one of two powder sugar protocols. The first is described in Aliano et al. (2005) in which bee repellent (Bee Go®) is used to drive adult bees into a box measuring 46.4 cm x 41.3 cm x 30.5 cm. The bees are then dusted with powder sugar inside the box. The second method involves dusting the tops of all frames with powder sugar and collecting mites as they fall through screen bottom boards onto plastic boards placed under the colony. Living mites were brought into the lab and housed on water-moistened filter paper suspended inside a clean, quart-sized glass jar. Jars with mites were maintained in an incubator at 32° C and ~40% relative humidity while marking was completed. Marking was accomplished the same day mites were collected. All mites were marked with correction fluid, using the protocol described in Kirrane et al. (2012). After marking, mites were inoculated onto cells containing ten-day-old honey bee larvae (see below).

Preparing honey bee worker larvae for inoculation

Ten days prior to mite inoculation, queens from four test colonies were individually caged on an empty drawn deep comb for 24 hours to ensure uniform age of developing larvae. Queens were moved to a new frame every 24 hours; each frame was labeled with the date eggs were laid. This was done for four consecutive days.

Frames with ten-day-old larvae were removed from their colonies; adult bees were brushed off and brought back to the UGA Honey Bee Lab for inoculation. A scalpel was used to make a slit in the capping, a marked mite was placed inside the cell, and the

slit was gently pushed back into place. A sheet of transparency film was used to map the inoculated larvae to aid in mite recapture. Frames were returned to the parent colony immediately after mite inoculation. This procedure was performed for each of the four test colonies. Inoculations continued for four days and were performed in a darkened room at a temperature of ~32° C at ~40% relative humidity, to improve survivorship of mites and honey bee larvae. Red lights were utilized to minimize bee stress.

Mites were re-collected 10 days after inoculation when bee larvae were 20 days old. Frames were removed, adult bees brushed off, and the frames brought back to the UGA Honey Bee Lab. With the aid of the mapped transparency films, cells of inoculated brood, now pupae, were manually uncapped with forceps. All cells containing marked mites and their offspring were collected for experimental trials.

Only marked post-partum mites and their unmarked pre-partum daughters were used in the study. Unmarked pre-partum mites are assumed to have never reproduced while marked post-partum mites are assumed to have reproduced at least once. Only mites originating from a cell with a marked mite were used in the study. To control for honey bee larvae that may have already contained a post-partum mite in addition to the marked inoculated mite, each mite was inspected carefully, and only the marked mite as well as any mites that were obviously lightly sclerotized, were used. No unmarked, darkly sclerotized mites were mistakenly used as pre-partum mites.

The two cohorts of mites were placed on water-moistened filter paper suspended inside pint sized glass jars and placed in an incubator at 32° C and ~40% relative humidity. Mites were used in trials the day of collection.

Collecting worker honey bees

The same four colonies that produced pre- and post-partum mites were used as source colonies for adult honey bees. Mite-free, newly emerged teneral workers (NEW) found when searching for marked mites were used as NEW bees. Pollen foragers were collected directly off the comb; only bees with pollen in their corbicula were used.

Each of the four test colonies was positioned at least 3.2 km from each other or any other known colony to minimize the chance of bees drifting between the four colonies.

Bees were housed in new Ziplock® plastic containers with air holes and provided 1:1 sugar water. They were held in an incubator at 32° C and ~40% relative humidity until used. Bees were used within 24 hours of collection. Bees were immobilized with CO_2 for placement into Petri dishes and examined for phoretic mites as they were being added. Bees found with phoretic mites were not used.

Mite trials

Mite choice trials were conducted over four consecutive days utilizing a different mite source colony each day. Each trial replicate consisted of a Fisherbrand® 100 x 15mm Petri dish containing one mite and three bees. The mite was given a choice of three bees (Table 3.1): (1) a homocolonial NEW bee, (2) a homocolonial pollen forager, and (3) a heterocolonial pollen forager from one of the other three test colonies. Each combination was replicated three times.

Table 3.1 Experimental design for live mite host choice assay.

	Honey Bee Colony Number			
Honey Bee Colony Number	1	2	3	4
1	.	NEW_1 F_1 F_2	NEW_1 F_1 F_3	NEW_1 F_1 F_4
2	NEW_2 F_2 F_1	.	NEW_2 F_2 F_3	NEW_2 F_2 F_4
3	NEW_3 F_3 F_1	NEW_3 F_3 F_2	.	NEW_3 F_3 F_4
4	NEW_4 F_4 F_1	NEW_4 F_4 F_2	NEW_4 F_4 F_3	.

NEW = newly emerged homocolonial bee
$F_{\text{col number}}$ = pollen forager

Bees were immobilized with CO_2, inspected for phoretic mites, and once deemed mite free, placed equi-distance from each other around the sides of the Petri dish. Bees had either their right, left, or both forewings clipped for cohort identification. Clipping occurred just prior to being placed in the Petri dish. Petri dishes were placed on tables in

a darkened room outfitted with red lights to minimize disturbance. The room was maintained at 32° C and ~40% relative humidity during trials. Petri dish trials followed procedures from Kuenen and Calderone (1997). Mite location was recorded every 15 min for four hours. If a mite or bee died before the four hours ended, data was no longer taken for that dish.

Statistical Analysis

Analysis of mite choice over time used a multinomial mixed effect model performed by the software package SAS/STAT® v. 9.3. A GLIMMIX procedure recognizing bee type as response variable, individual mite as random effect, and the continuous variable time as fixed effect. Preliminary analysis showed that on average both pre- and post-partum mites favored newly emerged worker bees over other bee choices; therefore, probabilities for a mite's presence on any bee type were calculated for each time point relative to the probability of the mite being on a new bee.

Results

The fixed effect of time on mite choice was significant ($F_{2,609} = 14.74$, $P < 0.0001$). Bee type was not significant ($F_{2,110} = 0.56$, $P = 0.5712$), but the interaction between time*type was significant ($F_{2,609} = 5.03$ $P = 0.0068$).

Over time, the probability of a post-partum mite choosing a heterocolonial pollen forager decreased significantly when compared to the probability of choosing a newly-emerged worker (t(df=609) = 3.03, $P = 0.0025$). A comparison of probabilities between post-partum mites choosing between homocolonial pollen foragers and newly emerged workers found no significant decrease in mite choice over time (t(609) = 1.57, P =0.1163 (Fig. 3.1)).

The probability of a pre-partum mite being phoretic on a heterocolonial pollen forager decreased significantly compared to newly-emerged workers ($t(609) = 3.40$, $P = 0.0007$). The probability of a pre-partum mite being on a homocolonial pollen forager also decreased significantly compared to newly-emerged workers ($t(609)$, $P = 0.0002$).

Comparing the probability of mite choice over time between pre- and post-partum mites revealed that the probability of a pre-partum mite choosing a pollen forager (using newly emerged worker as a baseline) decreased significantly faster than for post-partum mites. This was true for both heterocolonial pollen foragers ($t(609) = 1.94$, $P = 0.05$), and homocolonial pollen foragers ($t(609) = 2.82$, $P = 0.005$ (Fig. 3.2)).

Discussion and Conclusions

Our results suggest subtle differences in risk tolerance between pre-partum and post-partum mites – an effect predictable in the context of differences in dispersal risk tolerance mediated by reproductive value. Pre-partum mites, with comparatively higher reproductive value, exhibit low risk tolerance and have a higher affinity for parasitizing "safe" house bees. In contrast, post-partum mites, with comparatively lower reproductive value, show a greater tolerance for "riskier" forager bees. It should be noted that on average both pre- and post-partum mites prefer young bees to forager bees; significant differences reported here focus on the comparative tolerance for risk expressed between the two mite types over time.

Post-partum mites exhibit a drastic reduction in reproductive success following the first reproductive cycle (DeRuijter and Calis 1988; Fries et al. 1994); this reduction means the relative costs for risky behavior is reduced in post-partum mites. As the mite's reproductive fitness decreases, risky dispersal behavior could be selected for because the

reward of emigrating to an unexploited resource could be large, in this case, uninfected honey bee brood – a virtually limitless reproductive resource for the risk-taker's progeny.

Acknowledgements

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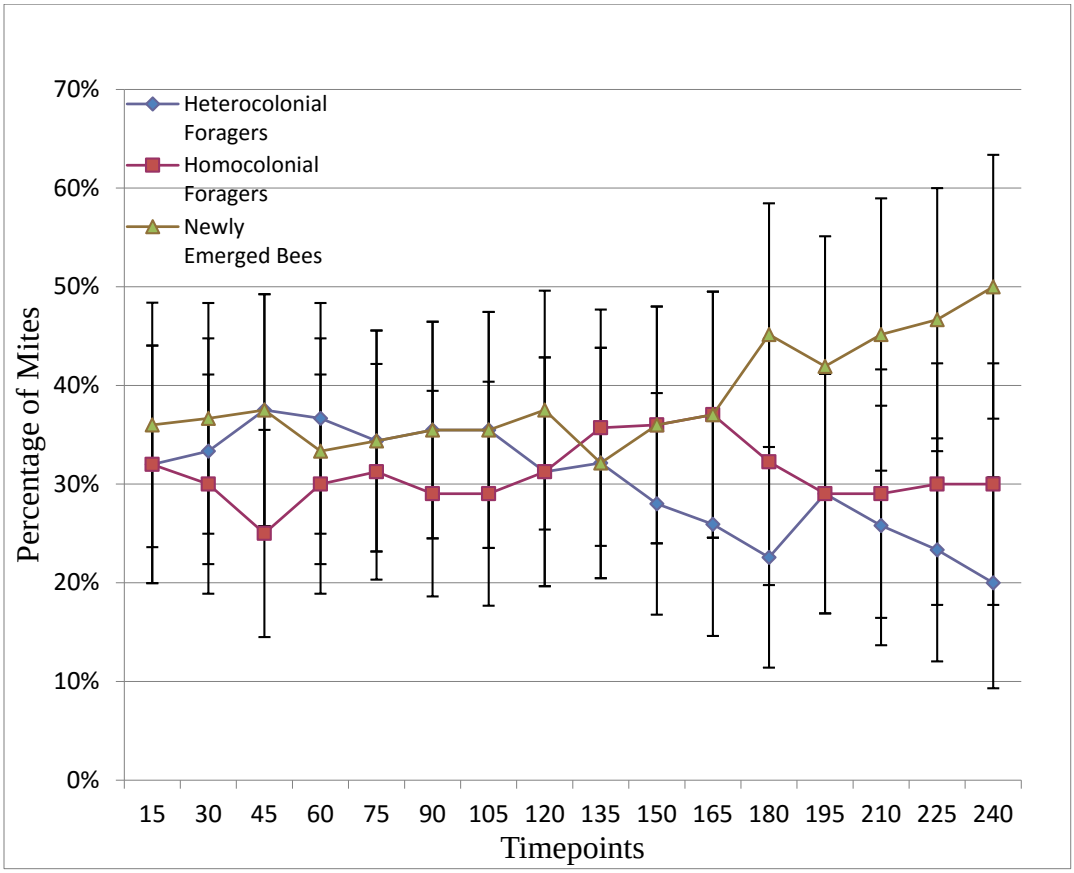

Figure 3.1 Percentage of post-partum mites on different bee types over 240 minutes. Relative to newly emerged worker bees, the probability of a post-partum mite being on a heterocolonial bee decreased significantly over time ($P = 0.0025$). Relative to newly emerged workers, the probability of a post-partum mite being on a homocolonial forager decreased, but not significantly ($P = 0.1163$). Error bars are SE of the mean.

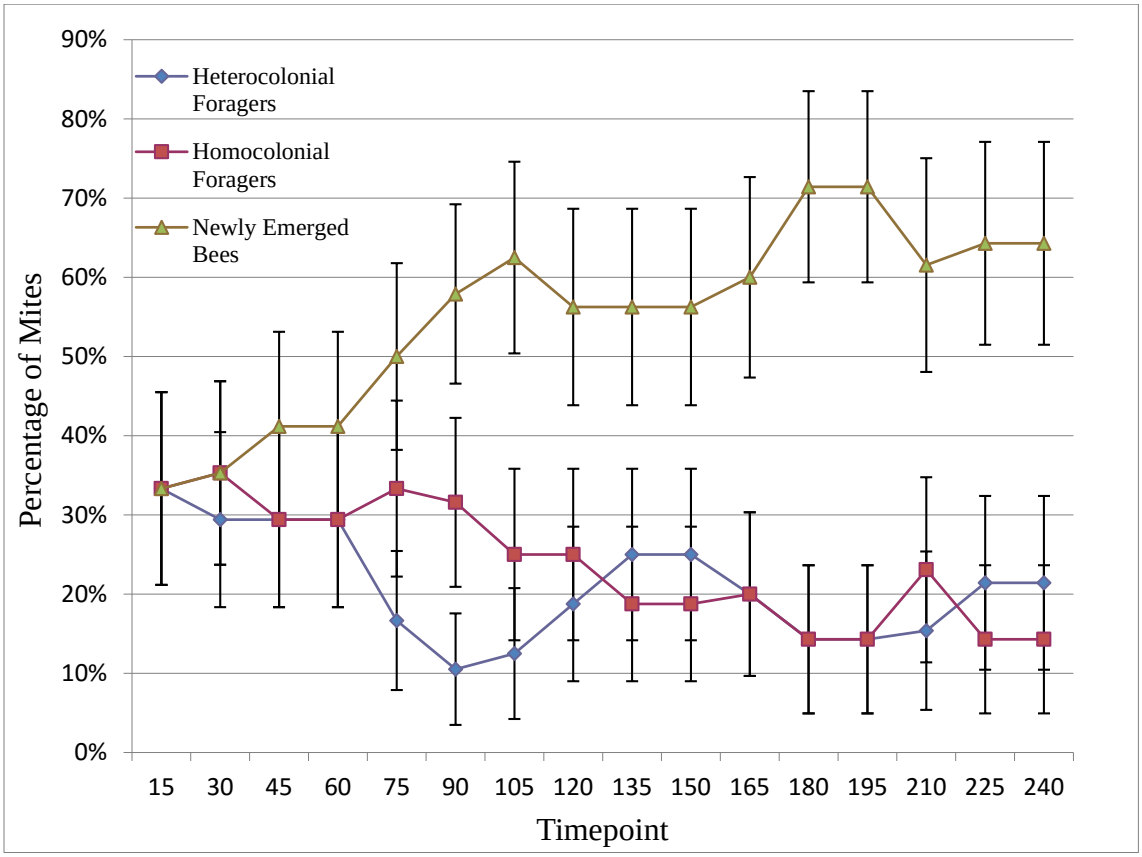

Figure 3.2 Percentage of pre-partum mites on different bee types over 240 minutes. Relative to newly emerged worker bees, the probability of a pre-partum mite being on a heterocolonial forager bee decreased significantly over time ($P = 0.0007$). Relative to newly emerged workers, the probability of a pre-partum mite being on a homocolonial forager decreased significantly over time ($P = 0.0002$). Rates at which pre-partum mites migrated off heterocolonial source forager bees ($P = 0.0533$) and homocolonial source forager bees ($P = 0.005$) were faster than post-partum mites. Error bars are SE of the mean.

CHAPTER 4

INFLUENCE OF QUEEN MATING NUMBER ON NESTMATE RECOGNITION AND COLONY FITNESS IN THE HONEY BEE *APIS MELLIFERA* [1]

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Abstract

Nestmate recognition is of vital importance to maintaining colony integrity in social insect societies. Among honey bees (*Apis mellifera*), the condition of polyandry, or multiple matings, increases intra-colony genetic diversity and may play a role in nestmate discrimination. Additionally, recent evidence suggests a positive relationship between genetic variance and colony fitness. Using instrumentally inseminated queens, we created two classes of colonies; one class contained queens inseminated with the sperm of 5 different drones, and the other contained queens inseminated with the sperm of 15 drones. Colonies were grouped into 12 pairs, with each pairing consisting of colonies of the same class. The number of heterocolonial workers, recognized by colony-specific marking, was used as a proxy measure of drift and heterocolonial worker acceptance. Our results found no significant difference in drift and worker acceptance between colony classes based on intra-colony genetic variance. Besides drift, we measured metrics of colony strength, parasite resistance, comb building, and forager recruitment as evidence of the benefits of genetic variance to colony fitness. No significant differences were reported in any of the categories except forager recruitment. In the 5 drone class colonies, significantly more nectar was collected per 100 bees than the 15 drone class colonies. Our data suggests that colony-level benefits attributed to genetic variance are not detectable between the 5- and 15-drone inseminated levels, as this range is within natural mating numbers. This is consistent with models predicting colony level benefits resulting from non-additive interactions among subfamilies and the accumulation of rare alleles under higher-than-predicted or hyperpolyandrous conditions.

Introduction

Worker bees display the altruism that supports social insect behavior; specifically, they relinquish their ability to reproduce in favor of helping a related individual reproduce (Breed 2014). To ensure that its efforts are directed at related individuals, an altruistic organism must be capable of kin recognition; it must possess the ability to ascertain relatedness (Hamilton 1964a, 1964b) and discern more-related individuals from less-related ones (Breed 2014).

Meanwhile, honey bee queens are polyandrous, mating with an average of 12±6.3 drones each (Tarpy et al. 2004). As a result, varying degrees of relatedness exist in a single colony. Polyandry lowers intra-colony relatedness originally needed for the evolution of altruism and affects inter-colony recognition cues used to distinguish nestmate from non-nestmate (Ratnieks 1991).

Nestmate recognition is of vital importance to maintaining colony integrity among social insects (Wilson 1971; Hölldobler and Wilson 1990; Downs and Ratnieks 1999) as well as preventing parasites and predators from entering the group (Breed 2014). Honey bees are vulnerable to the negative effects of drifted workers (Pfeiffer and Crailsheim 1998) and robbing bees (Free 1954), making discernment of nestmates from non-nestmates crucial. While the evolutionary mechanisms shaping kin selection via kin recognition are important, they need not be linked to cues used in inter-colony discrimination via nestmate recognition (Breed 2014).

The most widespread nestmate recognition system utilized in eusocial insects involves environmentally derived phenotypes which give all members of the colony a commonly held odor (Breed 2014; Breed, et al. 2015; Buckle and Greenberg 1981;

Downs and Ratnieks 1999; Page Jr, et al. 1991; Ratnieks 1991). Odor cues are derived from nest material (Breed, et al. 1998), food sources (Richard, Hefetz et al. 2004), the queen (Breed et al. 1992), genetic bases (Breed 1983; Breed et al. 1988; Breed et al. 1995; Greenberg 1979), or combinations of these (Downs and Ratnieks 1999; Moritz and Neumann 2004). In honey bees, odor cues originating from cuticular hydrocarbon profiles are of particular significance in nestmate discrimination (Breed and Stiller 1992). These hydrocarbons are at least partially under genetic control (Page Jr, et al. 1991). Assuming a component of the nestmate recognition system in honey bees is under genetic control, then polyandry and its resulting genetic variance has the potential to affect it.

Polyandry, and the positive effects of genetic variance on honey bee colony fitness, have been the subject of a string of recent papers (Delaplane et at. 2015; Mattila and Seeley 2007; Seeley and Tarpy 2007; Tarpy et al. 2013; Tarpy and Seeley 2006). The queen's mating number has been shown to associate positively with numerous measures of her colony's fitness, including disease resistance (Seeley and Tarpy 2007; Tarpy and Seeley 2006), weight gain, population growth (Mattila and Seeley 2007), improved brood rearing (Delaplane et al. 2015), lower Varroa mite infestation rate (Delaplane et al. 2015), and improved survival (Tarpy et al. 2013). The proceeding evidence lends support for the genetic variance (GV) model of how inclusive fitness is benefited by polyandry.

The GV model provides multiple hypotheses as to how inclusive fitness is realized through low intracolonial genetic relatedness (Fuchs and Moritz 1998). Some of the more generally accepted are: (i) reducing the risk of producing diploid males (which are sterile and normally killed by workers) (Page 1980); (ii) providing beneficial non-additive interactions among subfamilies (Crozier and Consul 1976; Oldroyd and Fewell

2007); (iii) reducing the likelihood that parasites or pathogens will decrease worker populations to such a level that threatens colony survival and reproduction (Sherman et al. 1988); and (iv) increasing the colony's ability to respond to environmental stability by "averaging out" extreme phenotypes (Page Jr. et al. 1995). Furthermore, recent evidence supports the relationship between GV, fitness, and evolution (Oldroyd and Fewell 2007). One area in which evidence is lacking is the affect of genetic variance on nestmate recognition system in honey bees.

In the present study, I utilized instrumentally inseminated queens to control colony polyandry levels, enabling comparison of nestmate recognition in response to intra-colony genetic variation. Mating frequencies of 5 or 15 drones were used, as these numbers bracket the natural mating numbers of 12±6.3 (Tarpy et al. 2004). Observing the incidence of heterocolonial members present in colonies with different degrees of intra-colony genetic variance allowed us to test the extent to which genetic variance, via polyandry, affects the discriminatory power of honey bees as well as their tolerance of heterocolonial workers. In addition to nestmate recognition, we measured metrics of colony strength, parasite resistance, comb building, and recruitment to a food source as evidence of the benefits of genetic variance to honey bee fitness.

Materials and Methods

Apiary Layout

This study was conducted from June-November 2014 in Athens, Georgia, USA (33.9500°N, 83.3833°W) and consisted of 25 honey bee colonies, all located in the same apiary. A diagram of apiary layout is given in Figure 4.1. Colonies were paired into 12 groups, with each pair containing colonies headed by queens inseminated with the same

number, either 5 or 15, of drones. Due to the uneven number of colonies, one lone colony, containing a 5-drone inseminated queen, stood alone and was included only in fitness measures. Within each pair, the colonies were spaced 0.5 m apart, and both colony entrances faced the same randomly-assigned cardinal direction.

Queen Insemination Procedure

All test queens were sisters produced from local stocks managed by the UGA Honey Bee Lab. Semen was derived from drones of randomly-selected source colonies also managed by the UGA Honey Bee Lab; these colonies were separate from the colonies used for queen sourcing. Drones were collected from 5 different colonies. The five-drone group received the semen from one drone of each colony, while the 15 drone group received the semen from 3 drones of each of the same 5 colonies. The semen from five drones was combined and diluted with 50% saline to increase semen mixing; this solution provided sufficient material to inseminate two queens with 8µl solution each. The process was repeated, utilizing different drones for each queen pair, until all queens of the five-drone group were inseminated. Next, the semen from 15 drones was combined, and likewise diluted with 50% saline.

Colony Strength, Drifting, Comb-Building, Varroa Mite Population, and Recruitment Measures

Colony strength data were collected for each colony in order to determine effects of polyandry on metrics associated with colony health. Total adult bee population, capped worker brood, and capped cm^2 honey were estimated on 29 Aug, 8 Oct, and 12 Nov 2014 using methods given in Section 4.2 in Delaplane, et al. (2013).

Drifting was measured by observing marked bees in each colony. Young bees, identified by their fuzzy appearance and inability to fly and sting, were collected and marked in the field. 100 young bees per colony were marked on the thorax; each colony received a distinct color combination using Testors® model paint. Two cohorts of 100 bees were marked 3 weeks apart to extend the study duration.

On the sixth night following bee marking, colonies were closed to ensure all bees were accounted for. Initial drifting measurements were taken the next day. Colonies were tented during inspection to minimize aggravated drift and robbing. All frames and hive body parts were inspected for marked bees. Colonies were inspected in the same manner, biweekly, in Sept and Oct 2014.

The comb-building ability of each colony was assessed during the study using the methods of Matilla and Seeley (2007). Empty deep frames were fitted with a 2.5-cm starter strip of wax foundation. One frame was placed in the middle of the brood nest of each test colony. Colonies were fed 1:1 sugar/water to stimulate comb building. Frames were left in the colony for 5 days before being removed to facilitate the measurement of new comb in cm^2. This data allowed estimation of cm^2 per adult bee.

Counts of the parasitic mite *Varroa destructor* were made twice during the study using sticky screen bottom board counts. Boards were inserted on 20 Aug and removed 25 Aug; another measurement was performed 6-10 Oct. Mites reproducing in brood were estimated on 5 Sep, 8 Oct, and 12 Nov by uncapping 100 worker bee brood cells per colony and inspecting the cells and pupae for mites. A total mite in brood measure was obtained by multiplying the percent of infested brood cells by the total capped brood estimates acquired from strength assessments. Lack of brood on the 12 Nov assessment,

due to natural shut down in brood production, caused us to exclude this sampling date from analysis. Phoretic mite populations (mites on adult bees) were estimated on 12 Nov using the alcohol wash method (~300 adult bees) (Dietemann et al. 2013). Total adult bee and total capped brood measurements enabled us to estimate colony mite populations.

Recruitment to a novel food source was measured twice during the study. On 21 Oct and 11 Nov, deep frames of empty, white, drawn comb were weighed (g) and inserted into each colony adjacent to the brood area. This was done to visualize dyed syrup (see below).

Three feeders were created using empty drawn frames placed within deep brood boxes; they were stationed in central locations within the apiary as depicted in Figure I. Each feeder was supplied with a mixture of 1:1 sugar/water solution, a small amount of honey to act as an odor stimulant, and food coloring. Bees were allowed to forage at the feeding stations for four hours before the frames were removed.

Both colored cells and non-colored cells on each frame were counted. The frames were weighed once more to obtain an ending weight. Subtracting ending weight from starting weight provided weight of all nectar collected. By multiplying the percent of colored cells by total nectar weight, we obtained the weight of color nectar gathered. Total color nectar gathered/total adult bee population (obtained from same method as comb-building assay) gave us color nectar collected in g per bee. This number is used as a proxy for recruitment. Negligible nectar was recovered from the 11 Nov date, and those data were excluded from analysis.

Statistical Analysis

Analyses were done with the Proc GLIMMIX or Proc MIXED procedures using SAS 9.14. Colony strength analysis used a Proc MIXED procedure recognizing adult bees, number of brood cells, and cm^2 capped honey as dependent variables, and insemination number as the fixed effect. Colony pair and date were set as random effects. Cm^2 capped honey was square-root-transformed due to skewness in the data.

Drifting analysis used a Proc GLIMMIX procedure recognizing insemination number as fixed effect with colony pair, cohort, and date as random effects. Due to many low counts in the data, a Poisson distribution was used.

The comb building analysis was measured by cm^2 constructed per 100 bees over 5 days. The analysis used a Proc MIXED procedure recognizing insemination number as fixed effect and colony pair as random effect. The data were log-transformed due to skewness in the data.

Sticky screen data were measured by mite drop per 24 hr by dividing number of mites recorded from sticky screens by five. Data were analyzed using a Proc MIXED procedure with insemination number as fixed effect and colony pair and date as random effects. The data were log-transformed due to skewness. Mites in brood were analyzed using Proc MIXED procedure for the 5 Sep and 8 Oct sampling dates with insemination number as fixed effect and colony pair and date as random effects. Due to many low counts in the data a Poisson distribution was used. Due to lack of brood the 12 Nov data were excluded from the analysis. Phoretic mite populations were analyzed using a Proc GLIMMIX procedure for measurements taken on 12 Nov with insemination number as fixed affect and apiary pair as random effect. Due to many low counts in the data a

Poisson distribution was used. Total colony mite counts were obtained from combining total phoretic and total mites in brood for the 12 Nov sampling date. Total mite counts were analyzed using a Proc GLIMMIX procedure with insemination number as the response variable and colony pair as random effect.

Recruitment data were measured as mg dyed syrup gathered per 100 bees per hr. Colony populations were estimated from colony strength data. Data were analyzed using a Proc MIXED procedure with insemination number as fixed effect and pair as random effect. Due to lack of data during the 11 Nov sampling date, only the 21 Oct date were included in the analysis. Data were log-transformed to account for skewness.

Results

Colony strength measurements were not significantly different among insemination groups, adult bee population (P=0.4983), capped brood (P=0.6352), or cm^2 capped honey (P=0.0725). The number of drifted bees was unaffected by insemination number (P=0.4346). Comb building ability (cm^2 per 100 bees) was not different between insemination groups (P=0.3093).

Among the three measures of Varroa mite population measured, no differences between insemination groups were detected: sticky screens (P=0.8823), phoretic mites (P=0.5885), mites in brood for 5 Sep and 8 Oct sampling dates (P=0.1041), and total mite population 12 Nov sampling date (P=0.5693).

The ability of colonies to recruit foragers to a novel food source was significantly different between insemination groups (P= 0.0429) with colonies headed by queens inseminated with 5 drones gathering, on average 5.66 g syrup/100 bees/hr and colonies headed by queens inseminated with 15 drones gathering 1.04 g/100 bees/hr.

Discussion and Conclusions

Our results failed to show a relationship between intra-nest genetic diversity and nestmate discrimination. The number of heterocolonial worker bees found among the 6 pairs of colonies headed by queens instrumentally inseminated with the mixed semen of 5 drones were not significantly different (P=0.4346) than the number of heterocolonial worker bees from 6 pairs of colonies headed by queens instrumentally inseminated with the mixed semen from 15 drones. These result support Downs and Ratnieks (1999) in sustaining the eminence of environmental cues over heritable cues in nestmate assessment. Only under controlled lab conditions have honey bees been able to discriminate between nestmates based on genetic differences (Breed 1983; Breed et al. 1988; Breed et al. 1995; Greenberg 1979; Moritz and Neumann 2004). Fittingly, our results failed to find a relationship between genetic variance and nestmate recognition in an apiary setting with a range of polyandry between 5 and 15 drones.

Our data also failed to detect an effect of genetic variance on colony strength as measured by adult bee population (P=0.4983), capped worker brood area (P=0.6352), or cm^2 of capped honey (P=0.0725). However, cm^2 capped honey approached significant levels, mirroring the results by Neumann and Moritz (2000) who found the impact of polyandry to have only a weak affect of honey yields and colony size.

We found no significant differences (P=0.3093) between treatment groups in comb building ability. This supports the findings of Delaplane et al. (2015) in which colonies headed by instrumentally inseminated hyperpolyandrous queens (15, 30, or 60 drones) were tested. However, our results contrast with those of Mattila and Seeley

(2007) who reported a positive effect of comb building as a result of increased genetic diversity comparing colonies with a queen mating number of 1 drone and 15 drones.

Our data suggest that genetic variance had no measurable effect on levels of Varroa mite infestation as measured by sticky screen counts (P=0.8823), mites in brood (P=0.1041), mites on adult bees (P=0.5885), or total mite levels (P=0.5693). These results support Neumann and Moritz (2000) who found that an increase in polyandry had a weak effect on Varroa mite infestation; however, when comparing a range of polyandry between 15 to 60 drones, Delaplane et al. (2015) reported colony-level reduction in Varroa mites at ≥ 30 drone levels

Forager recruitment was affected by colony genetic variance (P= 0.0429); however, our data suggest no improvement with increasing polyandry and contrast with the the findings of Mattila et al. (2008) where signaling via the waggle-dance was increased, or Mattila and Seeley (2007) where comb building, brood rearing, weight gain, population size, food storage, and foraging activity were enhanced in colonies headed by 15-drone inseminated queens compared to single drone inseminated queens.

Overall our results suggest that an increase in genetic variance within the range described here does not affect nestmate recognition. This sustains other studies (Ratnieks 1991; Downs and Ratnieks 1999) which conclude that colony odors deriving from environmental sources are predominantly used in honey bee nestmate recognition. A nestmate recognition system based on genetically derived odors was not found at our two polyandry levels.

Our data suggests that colony level benefits attributed to genetic variance are not discriminated between the 5- and 15-drone inseminated level. These numbers are within

natural mating numbers. This supports previous data of Neumann and Moritz (2000), where naturally mated colonies (all queens mated with more than 10 drones) yielded no significant correlations between mating number and colonies size and honey yield.

Colony level benefits are realized at extreme ranges of mating numbers, either well below natural limits (1 and 15 drone) in Mattila and Seeley (2007); Mattila et al. (2008), or well above natural limits (15, 30, and 60 drone) in Delaplane et al. (2015). The latter is consistent with the hypothesis that rewards for high levels of polyandry are due to (1) non-additive genetic interactions among subfamilies, and (2) benefits afforded colonies through the capturing of rare alleles regulating resistance to pathogens.

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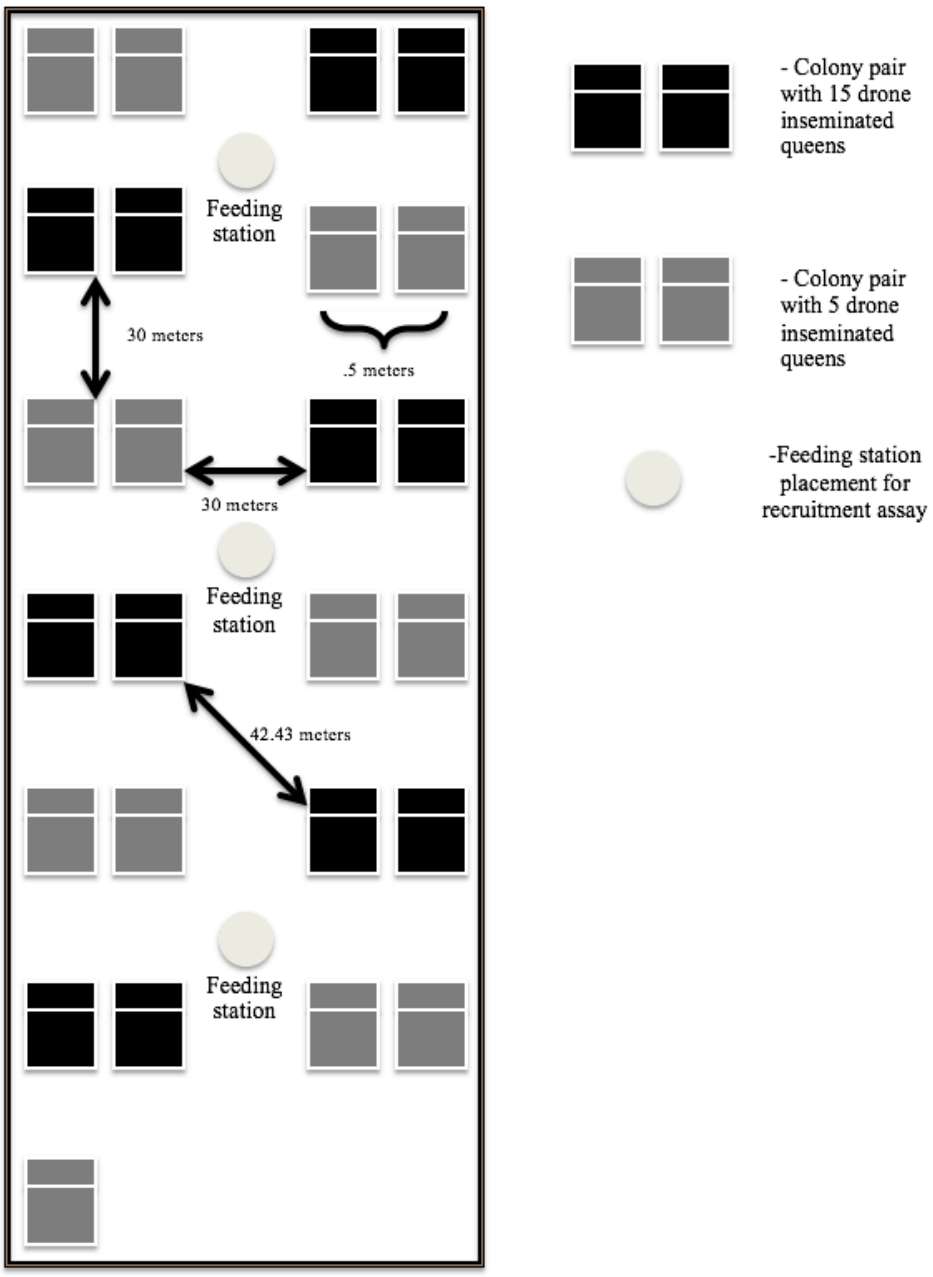

Figure 4.1 Apiary layout. Colony pairs were placed in two rows, with 12 colony pairs in each, and the additional stand-alone colony added to the end of the first row. The rows were 30 m apart, and colony pairs in each row were placed 30 m apart as well. As a result, each colony pair was at least 30 m from the next. Additionally, treatment pairs were alternated such that no similar pair was next to each other.

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CHAPTER 5

CONCLUSIONS

My research furthered understanding of the host/parasite relationship between *A. mellifera* and *V. destructor* by examining effects of inter-colony distance, mite host choice, and honey bee colony polyandry on horizontal transmission of the mite between colonies of honey bees. Collective examination and synthesis of these individual studies facilitate development of a multi-faceted approach to mite management.

My research on inter-colony distance uncovered a significant dynamic between inter-colony distance and Varroa mite population growth on a landscape scale (Chapter 2). As distance between colonies diminishes, drifting and robbing behaviors become more frequent, resulting in greater horizontal transmission of mites among honey bee colonies (Sakofksi et al. 1990; Greatti et al. 1992; Frey et al. 2011; Seeley and Smith 2015). Inter-colony movement supports mite reproduction at the apiary level by increasing access to and thereby decreasing competition for honey bee brood, which is otherwise a limiting resource.

In managed situations where apiaries may consist of hundreds of colonies with average inter-colony distances less than 1 meter, the addition of a single heavily infested colony has the potential to increase the Varroa mite population throughout the whole apiary. Colonies collapsing from heavy mite infestation are often robbed by nearby colonies, facilitating horizontal transmission (Renz and Rosenkranz 2001; Cervo et al. 2014). In my study, I found that apiaries with inter-colony distances of 100 m had

significantly fewer mites per colony than those with inter-colony distances of 0 or 10 m, expanding upon the previous findings of Sakofksi et al. (1990), Greatti et al. (1992), Frey et al. (2011), and Seeley and Smith (2015) regarding the relationship between inter-colony distance and horizontal transmission. These data suggest that beekeepers should increase inter-colony spacing to the extent possible to reduce drifting and robbing among colonies, mitigating both the spread and population growth of Varroa mites throughout the apiary.

In addition to studying the effects of inter-colony distance on horizontal Varroa mite transmission, I examined the relationship between mite reproductive value and risk tolerance (Chapter 3). Parasites depend on their ability to assess host conditions and disperse from an exploited host in search of new hosts (Cervo et. al 2014). Studying the factors affecting Varroa mite dispersal allows us to understand what drives horizontal transmission from the mite's perspective.

Research has shown that mites can use chemical cues to distinguish between nurse bees and foragers, and that they typically prefer to parasitize young nurse bees as these offer the greatest chance to contact more brood for reproduction (Del Piccolo et al. 2010). However, a study by Kuenen and Calderone (1997) identified a population of mites that preferred older pollen foragers. They postulated that this sub-set of mites might represent a population of mites in a dispersal phase. Later, Cervo et. al (2014) hypothesized that in situations where colonies are collapsing due to high mite infestation, some mites should adopt a dispersal strategy to abandon their dying hosts in favor of new ones. Dispersal behavior, while risky, has the selective advantage of offering the risk

taker a new host with lower mite numbers, thereby decreasing competition for honey bee brood in which to reproduce.

By applying life history theory which suggests a relationship between reproductive value and risk tolerance (Ghalambor and Martin 2001), I predicted that mites with different reproductive values would vary in their propensity to engage in the risky behavior of dispersal. My results support this hypothesis. I found that older mites with comparatively lower reproductive value are more likely than pre-partum mites to parasitize older pollen forager bees. Understanding which mites are more likely to disperse may provide a starting point for further investigation into mite control techniques.

The study of honey bee evolutionary biology, specifically polyandry, provides another angle from which we can identify management practices that best support colony health. A number of recent studies has shown a positive relationship between intra-colony genetic variance (GV), which is a function of queen mating number, and measures of colony fitness (Delaplane et al. 2015; Mattila and Seeley 2007; Mattila et al. 2008; Tarpy et al. 2013). However, the extent to which polyandry regulates other aspects of colony biology, such as nestmate recognition and parasite transmission, has been largely untouched in the literature.

My third research project examined the effects of queen mating number on nestmate recognition in honey bee colonies (Chapter 4). The ability to discriminate between nestmates and non-nestmates is important in controlling drift and robbing among honey bee colonies, the two major sources of horizontally transmitted mites.

Within a range of genetic diversity represented by queens inseminated with semen of either 5 or 15 drones, I could not detect differences in nestmate recognition nor other measures of colony fitness. It should be noted, however, that these mating numbers bracket those observed in nature (Tarpy 2004). Hyperpolyandrous colonies, those headed by queens inseminated with more than 30 drones, warrant further study, as past researchers have detected colony-level effects at such extreme ranges (Delaplane et al. 2015). Such research is necessary to determine the extent to which hyperpolyandry can be managed and applied for a holistic program of honey bee health management.

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