LIFE HISTORY PLASTICITY OF MANAGED HONEYBEES; BEHAVIORAL RESPONSE TO ENVIRONMENTAL CONDITIONS THAT PROMOTE OR ATTENUATE *VARROA DESTRUCTOR*

A Thesis By

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Abstract:
Colony Collapse Disorder (CCD) of managed honeybees has become a serious cause for concern within the agricultural sector. Research in recent years has reported *Varroa destructor* and other environmental stressors may be the foremost reasons for these increased losses. We developed this research towards gaining a more comprehensive understanding of adaptive honeybee behaviors that may attenuate *Varroa* mite populations and how environmental factors, such as humidity, play an integral role. There is a strong negative correlation (r = - 0.982, p < 0.01) between relative humidity and its effect on live *Varroa* mite populations. Increases in relative humidity increase the probability of observing allogrooming behavior in a series of logistic regression models showed significance, with one exception. Additionally, there is a moderate negative correlation between the total proportion of time spent grooming and the number of live *Varroa* mites (r = - 0.178, p < 0.05). The research conducted blends scientific methodologies and beekeeping experience to develop research methods that could help beekeepers explore *Varroa* presence in their apiaries and determine whether their honeybees are adapting behaviors that promote or attenuate mite populations.

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CHAPTER 1

INTRODUCTION

Colony Collapse Disorder (CCD) of managed honeybees has become a serious cause for concern in the last two decades. In recent years, the research has reported *Varroa destructor* and other environmental stressors may be the foremost reasons for these increased losses (Le Conte & Navajas, 2008; Le Conte et al., 2010; vanEngelsdorp et al., 2009). This research looks at gaining a more comprehensive understanding of adaptive honeybee behavior that may attenuate *Varroa* mite populations and the role environmental factors, such as humidity, play in mediating this behavior. The objectives of this research were to (i) determine the effect of weekly variation in external humidity on the presence of autogrooming and allogrooming behavior in our colonies; (ii) categorize the type of grooming and its observable frequency as it may pertain to altering time allocation patterns; (iii) categorize the frequency and grooming damage present; and (iv) conduct this research in real-time using an active hive that faces many natural constraints daily.

Human and Honeybee Associations

Humans and honeybees have had a long, mutualist relationship. For humans, honeybees have been an essential source of honey and beeswax throughout our history (Carson & Connor, 2013). Honey is one of the most energy-dense food sources that occurs naturally; for many hunter-gather societies, like the Hadza, it is a vital food resource (Marlowe et al., 2014). Hill et al. (1984) identified raw honey, from *Apis mellifera*, made up between 0.4-44% of the calories collected on different trips and consisting of 2,673 Calories/kilograms. Additionally, beekeeping is a long-used skill set in human history. Even hunter-gatherer societies, for example the Ache, build fires to smoke out the bees during honey pursuits (Hill et al., 1985). Beekeepers all over the world have developed indigenous knowledge that has been passed down from generation to generation. These methods persist today where communities have little access to external supplies (Abebe, 2011). The earliest record of apiculture comes from Egypt, where paintings on temples and tombs portrayed the importance honey played in spiritual practices, social activities, and economic activities (Carson & Connor, 2013). There are even references in the Bible and Qur'an mentioning beekeeping and the use of bees for pollinating crops (Carson & Connor, 2013). Traditional beekeeping is an essential component of agriculture and agroecology. In Southern Mexico, traditional methods employed planting techniques around their crops to entice honeybee visitation in an attempt to increase pollination efforts of particular plots (Dixon, 1987). Beekeeping is a crucial agricultural practice since honey is not only a food source but is used in medicines and natural remedies (Abebe, 2011; Muli et al., 2007). The importance of honey and beeswax even dates back to the sixteenth century, whereby honey from the Balsas River basin in the Yucatan peninsula was a form of tribute to the Triple Alliance (Aztec, Texcoco, and Tlacopan) (Dixon, 1987). Although honey production and bees are less likely to be used as a tribute nowadays, honeybee management continues to be a significant component of agricultural economies and biodiversity.

Mutualistic Relationship: Human and Honeybee

Managed European honeybees, *Apis mellifera*, are a vitally important part of the ecosystem and economy. "[Honeybees] are human companions of longstanding; our futures [will be entwined with one another]" (Phillips, 2014, p. 149). This notion is becoming even more apparent in the United States as our agricultural practices heavily rely on *A. mellifera* to pollinate 71 out of the 100 crops that provide 90% of the world's food source, as reported by Environmental California (2015) and EFSA (2016). In the United States, it is estimated that honeybees add approximately $17 billion to the agricultural sector per year, 35% of our specialty crops are dependent on honeybees for pollination, and an additional 84% of cultivated plants require some form of interaction with them (Le Conte & Navajas, 2008). Especially in California, honeybees assist annually in pollinating almond orchards. Science Daily (2019) reported that almond growers in Central California rented nearly 1.5 million colonies, valuing $300 million in honeybees, to pollinate their orchards. Almond production in California, directly and indirectly, contributes more than $21.5 billion to the economy and provides over 100,000 jobs annually (Sumner et al., 2014). Without the assistance of managed honeybees, the population of native bees is not high enough to meet pollination requirements at this scale. The result would be a significant loss in revenue for many specialty crops and the subsequent farmers.

In recent years, there has been an accelerating decline in honeybee populations reported worldwide, especially in the United States. Some of the factors contributing to these increased annual losses are loss of habitat (food source), climate change, increased parasitic infestations (*Varroa destructor*), and neonicotinoids. Estimates suggest crop yield dependent on pollinators will decrease by 90% without honeybees (Klein et al., 2007; Kremen et al., 2007). Although we do not classify managed honeybees as domestic animals, they have an intense reliance on humans to manage their environments for them to be successful. Nevertheless, in the wake of the industrialized agricultural movement, humans have not kept up on our end of this reciprocal relationship. The increased incentive of monoculture has decreased the food variety for honeybees. Many studies have found that when bees are being parasitized, those fed polyfloral diet blends lived longer than those fed a monofloral diet (Di Pasquale et al., 2013). The use of pesticides by farmers also results in the death of foragers and these individuals engage in one of the most critical tasks of bringing back food for the colony. The colony is highly dependent on foragers to supply all individuals with food (including the queen), and the forager status is already the most in danger in the caste system. Recent human activities and industrialization are making it even more so. Moreover, industrialization has prioritized yield over yield resilience and biodiversity through the usage of monoculture, nitrogen fertilizers, and insecticides. Shifting priorities, consistent with agroecology favors biodiversity and prioritizes the health of other species, such as honeybees. Agroecology is the science and practice of applying ecological principles to agriculture, this leads to the idea that treating farms and apiaries as ecosystems with the goal to mimic natural processes (Neff, 2014). More effort is needed to understand the complex factors that govern our agricultural practices, especially how our current practices influence the biotic and abiotic factors that affect honeybees (Altieri, 1989).

Varroa destructor

Parasites are especially detrimental to honeybee colonies, *Varroa destructor* being the most prevalent. Some reports attribute these losses to the increasing populations of *Varroa destructor*, formally known as *Varroa jacobsoni*, which switched to *A. mellifera* from its natural host (*Apis cerana*) during the 1950s (Anderson & Trueman, 2000). Since then, they have spread throughout most of the world at an unprecedented rate (Oldroyd, 1999). *Varroa* mites are now the most dominant pests of European honeybees and research links them with the worldwide collapse of millions of colonies (Martin, 1998). *Varroa* mites are ectoparasites that attach to the sides of honeybee pupae and adults in the brood cells and suck on the fat body tissue using their gnathosoma (mouthpart) to pierce the bee's cuticle (the fat body holds most of the mitochondria and enzymes) (Martin, 2001). There are two primary phases in the *Varroa* mite lifecycle: the female phoretic phase (attached to adult bees) (see Figure 1) and the reproductive phase (which takes place in the sealed brood cell) (Rosenkranz et al., 2010). The males and nymphal stages found in the sealed brood cells cannot survive outside the cells (Rosenkranz et al., 2010).

Due to *A. mellifera*'s lack of co-evolution, European honeybees have minimal to no resistance to *Varroa* mite infestations, resulting in devastating losses (Le Conte et al., 2010). The APHS National Honey Bee Survey (2017-2018) sent 24 kits per state, except California which received 48 (24 solely for bees used in almond orchard pollinations) to test for *V. destructor* presence (Fahey et al., 2019). Of these 947 alcohol samples taken, 848 tested positive for *V. destructor* (89.5%, 31.7% of the samples were over the threshold of 3/100 individuals) (Fahey et al., 2019). Moreover, increased infestation numbers can result in poor honeybee colony performance and even colony loss (Carneiro et al., 2007; Egekwu et al., 2018). The loss of the fat body and hemolymph can result in the honeybee being underdeveloped, ultimately having a shorter lifespan (Boecking & Genersch, 2008).

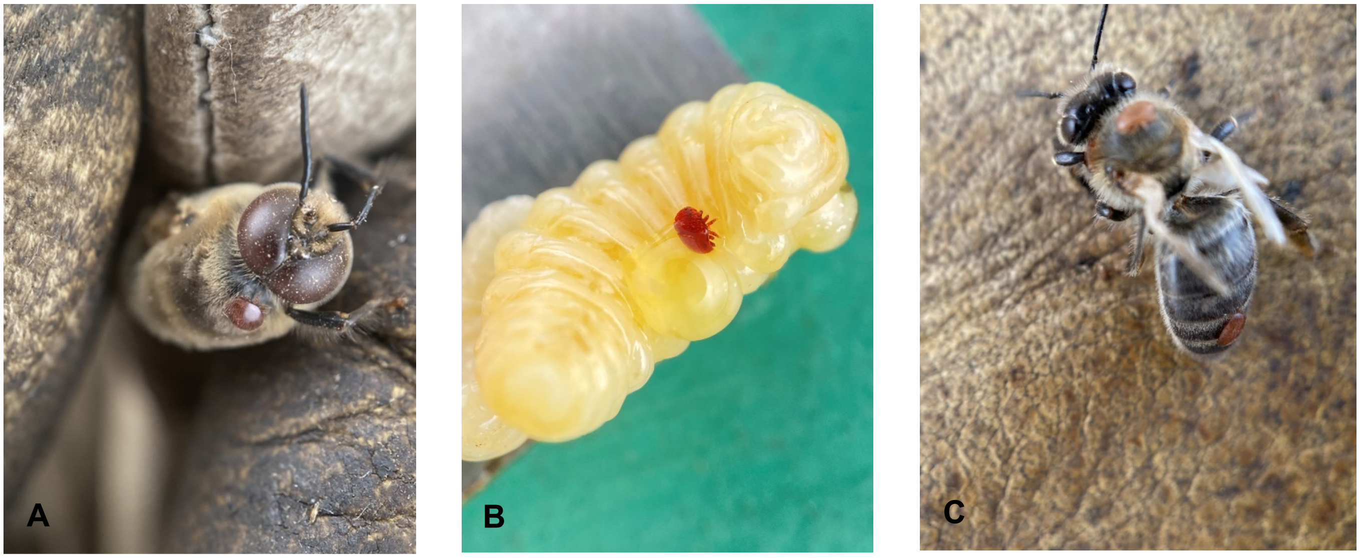


Figure 1. Female phoretic *Varroa* mite. **A.** A female phoretic *Varroa* mite attached to the thorax of a drone. **B.** A female phoretic *Varroa* mite on drone larva. **C.** Worker bee infected with deformed wing virus. These photographs were captured by Cal. State University, Fullerton Environmental Studies graduate student, Steve Anitcona, in October 2020 and March 2021 at the CSUF Arboretum Apiary.

With the growing rates of *V. destructor* populations and the increasing loss of colonies, many communities are frustrated. Los Angeles County Beekeepers Association (2019) reported they had lost 40% of their colonies in 2018. During the “overwintering months” in Southern California, beekeepers have experienced an increased loss of their colonies that correlated with increased pathogen levels that arise during colder climatic periods, even though Southern California experiences a much milder climate during these months than other regions of the United States (Ricigliano et al., 2018). Essential management practices require beekeepers to treat their colonies at the end of the winter period before spring, prior to the honey yield season. To delay treatments would allow the mite populations to grow during the spring and summer months, and with higher infestation rates, colonies are at a higher risk of collapsing (Giacobino et al., 2016). Similar to Western countries, traditional beekeeping practices are not without their troubles. There are reports of higher absconding events (swarming behavior), which could be due to drastic seasonal changes or higher parasitic infestations (Yirga et al., 2012). Unfortunately, there have been reports of *V. destructor* present in both Kenya and Tanzania that have a similar origin to the South Korean haplotype that is the most persistent throughout the world (Fazier et al., 2009). This indicates that *V. destructor* populations have spread worldwide, presumably found in every country except Australia (Rosenkranz et al., 2010).

Weekly *Varroa* mite inspections that follow the procedures of Barlow and Fell (2006) and Lee et al. (2010) are standard practices for beekeepers. These procedures obtain a count of live *Varroa* mites per 300 individuals which can indicate whether the number of parasites present is below or exceeds the threshold. Beekeepers must always be aware of the population growth rates of *Varroa* mites in their colonies. The increase of secondary infections caused by varroosis (like deformed wing virus) and the lack of comprehensive, coordinated treatment plans by beekeepers seem to perpetuate these losses (Boecking & Genersch, 2008). Beekeepers' lack of long-term experience treating *Varroa* mites, in conjunction with *Apis mellifera*'s lack of co-evolutionary host-parasite relationship, has resulted in the growing concerns for the future of honeybees (Rosenkranz et al., 2010). Even more concerning, some beekeepers report that traditional treatments, such as miticides, are no longer as effective as they once were. The lack of effectiveness of miticides has led the continuation of mite infestations without any method to control them. These beekeepers' concerns are similar to doctors' concerns about the rise of antibiotic-resistant bacterial strands due to over-prescribed antibiotics. Miticides are becoming overused, and hives often see multiple treatments throughout the year because of incessant *Varroa* mite infestations. Without these periodic treatments, honeybee colonies are expected to only survive 2-3 years after the infestation (Rosenkranz et al., 2010).

Many factors can affect the growth of *Varroa* mite populations. New research is being conducted that links higher wind speeds, lower humidity, and lower temperature to the increased prevalence rate of *Varroa* mites within colonies. Mitchell (2019) uses theoretical thermofluidic analysis to calculate models indicating that optimal internal humidity when reached could reduce the fecundity of *V. destructor*. In tropical regions, the limited growth of mite populations is attributed to the higher temperatures and extreme humidity reducing reproduction rates (Kraus et al., 1998; Kraus & Velthuis, 1997; Le Conte et al., 1990; Ritter, 1988 as cited in Harris et al., 2003). Harris et al. (2003) found a correlation between temperature and relative humidity with the mean growth rate of *Varroa* mites.

Their results showed a correlation between the annual mean growth rate and the number of days when the average relative humidity was ≤ 70% (Harris et al., 2003). The internal temperature can determine foraging activities, food consumption, and brood-rearing, all of which are important in determining the colony's overall health (Zacepins & Karasha, 2013). For honeybees, the central brood area's optimal internal temperature needs to be around 93-95º Fahrenheit (Southwick & Heldmaier, 1987). Monitoring temperature measurements, humidity, and weight can give honest indicators of the colony's health and the hive's capacity to maintain social homeostasis (Gil-Lebrero et al., 2017; Meikle et al., 2008; Southwick & Heldmaier, 1987; Zacepins & Karasha, 2013). Within the colony, honeybees manage their hive's internal temperature by contracting their flight muscles to generate sufficient energy that raises their body temperature a few degrees (Southwick & Heldmaier, 1987). When all workers cooperate, this maintains the needed temperature for optimal survivorship of the brood (Humphrey & Dykes, 2008; Southwick & Heldmaier, 1987). The number of cell-heating bees present determines the colony's ability to manage the internal temperature. That number of individuals affects the rate of time it takes to complete an absolute temperature change (Humphrey & Dykes, 2008).

Additionally, when honeybees are under several constraints, like high infestation rate and extreme temperature changes, reallocation of labor must occur to deal with at least one of the pressing issues (Li et al., 2018; Southwick & Heldmaier, 1987). Honeybee colonies are experiencing both biotic and abiotic stressors. Temperature and humidity are two of the most influential factors that affect the social homeostasis of the honeybees, influencing internal and external activities (Abou-Shaara et al., 2017). The colony's health is vital to completing daily tasks and managing the overall internal environment. Honeybees must coordinate and work together to best deal with the situation at hand, possibly deciding to mitigate one stressor and allowing the other to persist (like infestation rate). Due to these factors, honeybees are dependent on human management to help mitigate some of their environmental stressors.

Grooming

Honeybees express many behaviors that promote not only individual immunity but a social immunity, and individual bees cooperate in an attempt to mitigate pest infestations within the hive (Simone-Finstrom, 2017). Individual specializations in immune-related tasks, such as allogrooming, can strongly contribute to preventing the spread of pathogens and pests. Although there are tradeoffs between task specialization and efficiency when allogrooming was found to be a weak specialization, continued plasticity of other colony demands is all part of internal hive management (Cini et al., 2020). Grooming can be defined as a mechanism of social disease resistance behavior and described as a common mechanism for reducing ectoparasites in many organisms (Carr et al., 2020; Pettis & Pankiw, 1998). Grooming is one such specialization whereby honeybees use their legs and mandibles to remove debris and parasites. There are two types of grooming behavior observed in honeybees: auto-grooming (self) and allogrooming (grooming of other nestmates). Honeybees are natural groomers, like most animals; however, honeybees rely on multiple mechanisms for determining and removing debris or pests. Plasticity in honeybee behavior is adaptive because the flexibility of the behavioral response depends on the number of colony members engaged in the task (allogrooming) and the environmental factors present (*Varroa* mites) with the goal to increase reproductive success and survivorship of the colony (Cini et al., 2020; Simone-Finstrom, 2017).

Methods that influence grooming behavior may also be vital in reducing *V. destructor* prevalence rates. Pettis and Pankiw (1998) determined that grooming behavior changed based on tracheal mite levels. They found that as honeybees increased the initiation of the grooming dance and increased allogrooming the number of tracheal mites had increased, showing there is a correlation between increased parasitic infestations and increased behavioral response (Pettis & Pankiw, 1998). Several researchers propose implementing powdered sugar treatments to increase grooming behavior within the colony by eliciting group grooming responses (Aliano & Ellis, 2005; Berry et al., 2012; Stevanovic et al., 2012). Grooming behavior can be seen after a powdered sugar test has been performed after the 300 individuals have been returned to the colony. Land and Seeley (2004) studied grooming as a solicitation signal and documented the complexity of the grooming invitation dance. They discovered that when small particles, like powdered sugar, accumulate at the base of the wings, it triggers the individual to initiate the grooming invitation dance which is usually responded to by another bee coming to groom that individual (Land & Seeley, 2004). By initiating this innate behavior, honeybees can remove *Varroa* mites off their fellows through individual or group cleaning (Peng et al., 1987). A beekeeper who regularly performs powdered sugar tests on their colony has witnessed this and may dust the bees on brood frames to prompt grooming behaviors; this could be considered an integrated pest management strategy for treating *Varroa* mites (Berry et al., 2012). In adult honeybee laboratory trials, powdered sugar was tested to see its effect on the removal of *Varroa* mites. Fakhimzadeh (2001) found ninety-nine percent of the mites in the sugar treatment fell within 18 hours of the treatment beginning. This study indicated that powdered sugar may be an effective treatment for managing *Varroa* mites.

European honeybees, like their Asian counterparts, also partake in grooming behavior. Arechavaleta-Velasco and Guzmán-Novoa (2001) found that *A. mellifera* colonies showed more grooming behavior than previously given credit for in prior research. In *A. mellifera* colonies, more mites were reported falling to the hive floor. Of those fallen mites there was a high proportion of those that appeared to have been chewed, indicating a reduction in infection levels amongst the adult bees due to grooming (Arechavaleta-Velasco & Guzmán-Novoa, 2001). Damaged mites were observed with various losses from a single segment or appendages to the removal of all legs and the gnathosoma. Simultaneously, damage to the cuticle shell and scuta was rare (Ruttner & Hänel, 1992). The ability to suppress mite reproductive output would translate to lower fertility rates (Nganso et al., 2017). Fazier et al. (2009) reported that Africanized honeybees might exhibit higher degrees of hygienic and grooming behaviors than European honeybees. Hygienic behavior is associated with disease resistance, and reportedly the Africanized bees have been able to deal with these new *Varroa* mite infestations more effectively than their counterparts. Some studies argue that defensiveness, often described as aggressive behavior, is a colony signal expressing more diseaseresistant behavior. All beekeepers know Africanized bees are naturally more defensive than the traditional honeybees used in apiculture (Carr et al., 2020). It warrants further observational studies by beekeepers to determine if they noticed behavioral changes in their hive during periods of higher *Varroa* mite fecundity and whether this correlates with higher grooming practices.

Honeybee Life History Plasticity

Honeybees are successful eusocial animals that have impressive, inherent cooperative skills because of their unique social organization. Within a colony that consists of one reproductive queen, there can be between 20,000 and 60,000 workers and 10,000 to 30,000 at the brood stage (egg, larval, and pupae) (Martin, 2001). Individual workers within a social insect caste system display differing predispositions in the multitude of tasks they perform. They are often associated with physiological changes according to age (Flatt & Heyland, 2011). Polyethism is the division of labor present in the caste systems inherent in many social insect species. As their tasks change with age, the workers' glands will develop according to that corresponding task for honeybees (Vidal-Naquet, 2015). For example, nurses that process food have food-processing glands that are well developed during that period in their life but will decline when they switch to another task (Vidal-Naquet, 2015). There are five caste levels in a honeybee colony: the queen (reproductive female), plus four stages of workers (cell cleaning, brood nest, food storage, and forager) (Münch & Amdam, 2010; Seeley, 1982). The timing of a bee's life history milestone changes in response to interactions with their siblings throughout its life. For example, younger workers can become foragers earlier than expected if there is a lack of foragers present in the colony (Vidal-Naquet, 2015). Plasticity is an adaptive strategy because certain tasks, for example foraging, is high-risk and individual foragers face high mortality rates. A reduction in foragers corresponds to nectar and pollen shortages for the entire colony. This is detrimental to the survivorship of individuals and the development of the brood as a consequence. Flexibility in the timing of role transition has a high payoff for individuals and the colony as a whole. This plasticity is sensitive to features of the social environment, whereby the removal of a nurse bee can cause a forager bee to physically and behaviorally change back once again to become a nurse bee (Flatt & Heyland, 2011). The working caste in *Apis mellifera* represents the adaptive life history polyphenism (phenotypic plasticity) that helps conserve somatic functions (Flatt et al., 2013). It promotes survival during periods of environmental stress (i.e., *Varroa* mite infestations and environmental changes) (Flatt et al., 2013). By understanding critical elements of life history plasticity, beekeepers can follow individual bees through their various social tasks and read behaviors that may be indicators of plasticity that are apparent during these periods of environmental stress.

Colony Collapse Disorder

Colony Collapse Disorder (CCD) is a recent phenomenon whereby there is a rapid loss of worker bees in the hive (vanEngelsdorp et al., 2009). There is no definitive reasoning behind these increasing losses; however, beekeepers have an idea of some of the contributing factors that may result in colony collapses. Colony loss calculations and colony strength determinants are vitally important for beekeepers to understand the overall loss and the measures needed to manage the situation (van der Zee et al., 2015; vanEngelsdorp et al., 2009). Due to the ongoing losses of honeybee colonies, the rising concern of food security has become apparent in many political and economic discussions. CCD has left several apiaries in the United States with a reported annual loss of 30-70% of their hives, endangering the United States Agricultural Sector (vanEngelsdorp et al., 2009). Since 2006, honeybee colonies have reported astronomic losses in Europe and North America; these losses have been increasing every year (Le Conte et al., 2010). In 2008, the Farm Bill mandated research and required reporting on CCD to understand the causes, ways to strengthen colonies, and ways to improve agricultural profits (Andrews, 2019). Although there has been continued research in this area, there are still many unknowns regarding the main factors contributing to CCD.

Understanding all the factors and their interconnectivity that influence honeybee colony health and strength can help beekeepers understand the constraints on the colony. The honeybee colony health and strength model (see Figure 2) depicts the flow of arrows between the different factors and the influences they have on each other. Each factor has the capacity to influence colony health and strength directly and indirectly through the other factors. When honeybees experience periods of environmental stress, the constraints of dealing with multiple factors at once could lead to the increased losses of colonies. As suggested by vanEngelsdorp et al. (2009), the increase in *Varroa* mite infestations may not be the sole reason for CCD, but instead, it is the compilation of many factors as has been indicated in Figure 2. Many of the factors interact with one another and could be involved in the increase in colony losses, such as pesticides, parasitic loads, environmental conditions, and honeybee time allocation constraints (Le Conte et al., 2010). More research is needed to understand the complexity of each of these factors and where human management practices can come in to mitigate some of these stressors.

Social Science Perspective: Combining Differing Epistemic Forms of Knowledge

Colony losses are continuing to grow annually at an exponential rate, every year breaking the previous year's record, and beekeepers are perplexed about how best to respond. Since the early 2000s, we have heard phrases like "save the bees" and the "Anthropocene," eliciting concerns regarding species going extinct and the state of the world due to climate change. Within the beekeeping community, it goes beyond losing colonies annually. Beekeepers are calling into question the reliability of their profession and the sustainability of it in the future. As the primary stakeholders being affected by colony losses, something must change to incorporate beekeepers' various forms of knowledge that may be best suited for fixing this situation. There is a need to define new goals that go hand in hand with creating new landscape management plans geared toward the future, rather than focusing exclusively on current events (Marris, 2011). This new form of thinking in conservation connects the well-being of both humans, animals, and the environment (Marris, 2011). Many scholars call for a more-than-human approach, a new paradigm of human-nonhuman relationship, to be implemented for research and policymaking (Andrews, 2019; Haraway, 2008; Marris, 2011). The exclusion of non-scientists, like beekeepers, in policymaking and discussions about the future of the agricultural section are also being called into question (Adams, 2018; Phillips, 2014). Solving the multiple issues that place stress on honeybees that result in Colony Collapse Disorder requires more cooperation amongst the multiple stakeholders and our animal companions.

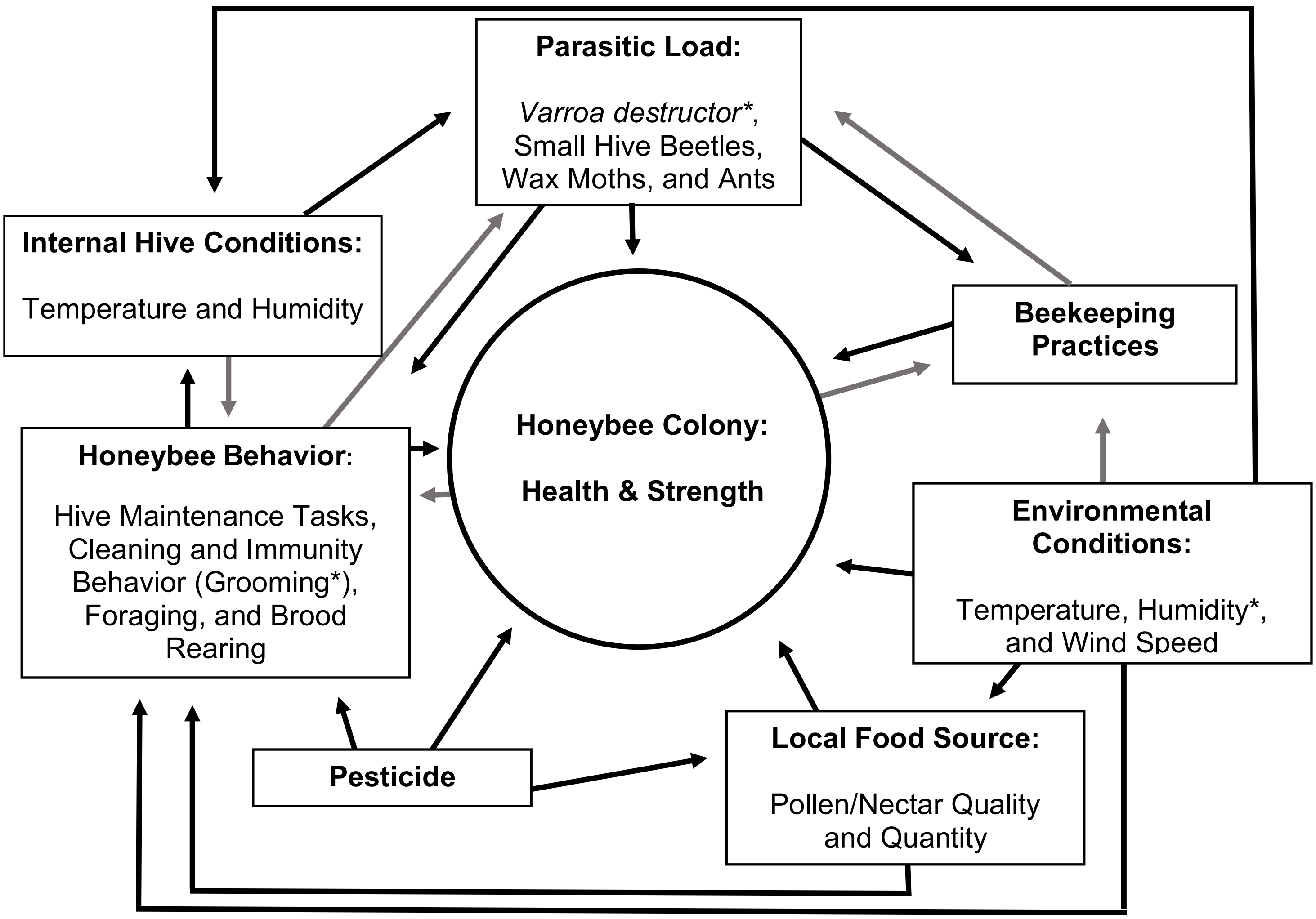


Figure 2. Honeybee Colony Health & Strength Model. This model outlines all the factors that can affect the health and strength of the colony. Each factor has the capacity to influence multiple factors that indirectly and directly affect the health and strength of the colony. For example, grooming behavior is directly influenced by pesticide exposure, parasitic load, and overall colony health. Grooming capability can also directly influence the parasitic load, whereby, directly and indirectly affecting the health and strength of the colony. Understanding each of these factors and how they interact with one another will help with solving the overarching problem of Colony Collapse Disorder (CCD).

Humans and animals are interconnected more than we think. Since the beginning of plant and animal domestication events, humans have changed much of our environment for our benefit. We have re-constructed our niches to make way for intensive industrial-agricultural practices and promised a “more is better approach.” Although, in light of climate change and the Anthropocene, these ways of thinking have been called into question. These significant manifestations of the

Anthropocene have made humans reconsider our consumptive behaviors and rethink our previous standards of life as it relates to “other-than-human-species” and a “more-than-human approach” as it pertains to bees (Phillips, 2014, p. 150; Weldemariam, 2020, p. 391). These new forms of dialogue argues that bees’ lives and humans’ lives are not viewed separately, but are matter-of-factly entangled (Phillips, 2014). Recent research into this paradigm switch teaches people how to “become with,” a practice that shifts humans’ focus onto a world perspective instead of individualistic (Haraway, 2008, p. 3). Not only do humans need to cooperate, but they need to begin cooperating with nature intrinsically.

Humans create their environment using what is known as Traditional Ecological Knowledge. Traditional Ecological Knowledge (TEK), consisting of a set of beliefs, knowledge, and practices that are handed down from generation to generation. Major proponents of TEK consist of individual’s knowledge of the time and location of resources in their environment (Smith, 2011). Members in small-scale societies can mentally construct seasonal habitat preferences and spatial distribution of elected plants and animals (Smith, 2011). Traditional Ecological Knowledge includes adaptations for the accumulation and transmission of this specialized knowledge throughout generations (Berkes et al., 2000). This knowledge can be used to interpret and respond to feedback in the environment that guide and directs resource management (Berkes et al., 2000). Traditional Ecological Knowledge refers to the evolving knowledge acquired by indigenous and local communities that have transcended hundreds, if not thousands, of years through direct contact with the land.

Humans have long before, during early tribal societies, cooperated with nature using TEK. Cristancho and Vining (2004) worked with the Letuama village from the Colombian Amazon. They learned this cultural group used ethical approaches and knowledge-based decision-making when it comes to human-nature interactions. This cultural group called it “natural justice,” whereby human and nature’s interactions are inherent, and the researchers argue this is a reciprocity argument. One of this group’s moral principles is: “[if] you give me something good or bad, [I will] give you back something good or bad” (Cristancho & Vining, 2004, p. 45). This argues that because nature provides humans with something, then in exchange, they must treat that with respect otherwise next time the outcome may not be as favorable. In this tribal society, they view their knowledgeable, usually an elder, as “keepers” and view them as “masters of nature” (Cristancho & Vining, 2004, pp. 42-44). Before taking from the land, one must ask the “keeper” first; the keepers then commune with nature and relays strict instructions for how much one can take from the land (Cristancho & Vining, 2004, pp. 42-44). In a sense, this is a strict conservation effort to prevent the tragedy of the commons. The concept of the tragedy of the commons is based on the idea that common-pool resources (CPR), without proper regulation or management, will be exploited by all community actors without regard for the cost imposed on other actors within the community, ultimately resulting in the overuse of the resource and leading to its inevitable depletion (Dietz et al., 2003; Hardin, 1968; Ostrom, 2002; Ostrom et al., 1999). When local communities are stable and have established institutions that are separate from outside forces, they can govern their resources sustainably for long periods successfully (Dietz et al., 2003). This conservation effort is not being practiced today in developed countries, especially not in the United States. It may be time to consider returning to this style of thinking and environmental planning.

The implementation of TEK in today’s agricultural system and beekeeping practices may help set us on a better path. New studies have shown that collaborations in local landscape research and the promotion of more inclusive partnerships may help bring TEK and the Indigenous and Local Knowledge (ILK) to the forefront of the research developmental process (Adade Williams et al., 2020). It is important, moving forward, to include more research to determine more effective ways to incorporate TEK and ILK with scientific knowledge to better study local landscapes and garner a clearer understanding of the social-ecological system (Adade Williams et al., 2020). Traditional beekeepers from Gurye-gun and Inje-gun South Korea possess a significant amount of knowledge about the biological characteristics of native honeybees, including their physical features and life cycles (Park & Yeo-Chang, 2012). Park and Yeo-Chang (2012) found that native beekeeping and their significant Traditional Forest Knowledge (TFK) showed signs of transmission and evolution throughout the generations. This means that these local communities have established knowledge centers that are not only being passed down to current generations, but these beekeepers are developing their beekeeping methods based on changes to their local environments. Guadilla-Sáez et al. (2019) conducted a similar study examining the changes in traditional management practices and the local perspectives on the impact of ecosystem diversity that led to the preservation of traditional farming systems in Spain. They were able to conclude that the combination of local and scientific knowledge of the ecological system was able to help them develop effective conservation measures regionally based on favorable practices that promote biodiversity and economic profitability.

For humans to cooperate with nature, it requires a great deal of effort to cooperate with ourselves first. Phillips (2014, p. 150) argues that this more-than-human approach has limitations and the relations of "heterogeneous elements" are apparent when you take into consideration beekeepers, agricultural productivity, biosecurity, and conservation. Many elements need to be considered and taken into account. Combining these different knowledge centers may help put many of these elements into perspective. This ultimately allows us to address the challenges of beekeeping, especially when considering the damage *Varroa destructor*, pesticides, and intensive monoculture practices have caused and their implications in the more significant issue of CCD. Phillip (2014) argues that by limiting the social science coverage of beekeepers and their knowledge, we are not understanding the bigger issues. By blending social practicing theories and more-than-human studies, Phillips (2014) brings attention to the interconnectivity and shared labor of beekeeping by arguing for the importance beekeepers should play when it comes to policymaking (Maderson & Wynne-Jones, 2016). Commercial beekeepers offer an alternative epistemic form of knowledge that utilizes different methodologies and observations and offers a different explanation for CCD (Kleinman & Suryanarayanan, 2013). This type of work and ethnographic research is essential for classifying beekeepers as stakeholders and showcasing the ways that their knowledge is beneficial for solving the many problems affecting bees.

Beekeepers and farmers can work together to build a more sustainable and resilient agricultural system. One recent idea is building hospitable corridors by improving conservation efforts at farms and promoting agri-environmental schemes (Marris, 2011, p. 141). Using multiple intercropping methods, farmers can promote wildlife and increase pollination by planting certain flowering plants around their farms. This mitigates the need to rent honeybee hives for certain seasons, as is done in Central California with almond orchards. There needs to be a shift within the agricultural sector, removing ourselves from thinking toward over-production and simply increasing farms' short-term revenue to one that is more sustainable and prompts a polyculture atmosphere. Agricultural and environmental policymaking creates hierarchal systems that often disregard beekeepers' knowledge and solely agree with scientists or large corporations (i.e., pesticide companies) (Maderson & Wynne-Jones, 2016). This ultimately results in a clash with pollinator conservation and limits sustainable agricultural practices that promote productivity (Maderson & Wynne-Jones, 2016). Nature and industry do not need to be viewed as mutually exclusive; what is better for nature will ultimately be better for the industry.

The interconnectivity between humans and honeybees is very different from any other human-animal relationship. The way we have constructed our niches to promote an industrialized agricultural system dependent on pollinators indicates that relationship. Our agricultural system's dependence on pollinators has manifested into our social and political lives with phrases like "save the bees," eliciting an emotional response to the situation at hand regarding the honeybee's disappearance. The characteristics of the human econiche are not just biologically or geographically based in nature but they are also socio-culturally based (Heft, 2007). Every aspect of how we construct our niches, especially in modern times, is linked to our social and cultural awareness. Furthermore, due to this new movement of understanding climate change and the Anthropocene, more voices advocate for a more-than-human approach in policymaking regarding how best to deal with honeybee losses.

Diverse thinking and knowledge centers are critical in understanding and solving environmental problems (Maderson & Wayne-Jones, 2016). The interactions between differing biotic and abiotic stressors could be responsible for the increased severity of colony collapses and the overall health of honeybees (Li et al., 2018). This research follows the principles of agroecology, utilizes differing epistemic forms of scientific and beekeeping experiences to conduct real-time observations, and gets a clearer understanding of external weather factors and *Varroa* mites' role in honeybee population declines. One of the main goals of this research is to join both beekeeping experience with science-based methodologies to find the causality between these various biotic and abiotic factors. Without a clear understanding of how intertwined humans and nature are within a reciprocal relationship, we will not be able to think about the environment using a more-than-human approach.

Research Hypotheses and Predictions

Research has shown the importance humidity plays on honeybees maintaining hive homeostasis and the role it plays on *Varroa* mite fecundity (Harris et al., 2003; Human et al., 2006). *Varroa* mites are partial to surviving and reproducing at lower humidity levels, which is why there tends to be a population increase during the overwintering months when humidity levels tend to drop below the optimal range researchers have defined. Currie and Tahmasbi's (2008) results showed that the environment's relative effectiveness of high- and low-grooming groups was affected.

Hypothesis 1 & Predictions

Hypothesis 1. If honeybees prioritize reduction of *Varroa* mites through allogrooming, then we expect more time devoted to this form of grooming during periods of lower relative humidity.

Prediction 1.1. During periods of low external humidity, we expect the time invested in body grooming to increase.

Prediction 1.2. As devotion to grooming activities increases, there will be an increase in the number of *Varroa* mites on the gridded sticky boards.

Prediction 1.3. Of these fallen individuals, the majority of mites will show signs of grooming damage.

Currie and Tahmasbi (2008) discovered mite mortality rates were greater in high-grooming groups of bees than in low-grooming groups when the temperature was at 77-93° Fahrenheit. The high mortality rate of adult female *Varroa* mites is vital in lowering reproductive rates (Nganso et al., 2017). Researchers observed the highest number of mutilated mites during May to September, which correlated with the decrease in *Varroa* mite populations (Mondragón et al., 2005). Because our location in Southern California has a milder winter climate, we are assuming that grooming rates will continue. A reduction in *Varroa* mite populations will correlate with an increase in the debris presence on the sticky board.

Hypothesis 2 & Prediction

Hypothesis 2. As humidity decreases, we expect the number of *Varroa* mites detected in a powdered sugar test to increase.

Prediction 2. There is a relationship between *Varroa* mite fecundity and relative humidity, and during periods of lower humidity *Varroa* mite fecundity should increase.

We know that *Varroa* mites have higher reproductive success during periods of lower temperature and lower relative humidity, which is why we expect to see population growth during our research period, before the overwintering months. The ultimate goal of this research is to determine alternative methods beekeepers could implement within their apiaries regularly that can help them gain a more in-depth understanding of their colonies' health. Moreover, these methods could help attenuate *Varroa* mite fecundity before their colonies are on the verge of collapse.

CHAPTER 2

METHODS

We conducted this research at the California State University, Fullerton Arboretum, where there has been an apiary since the 1970s. This research focuses on new colonies introduced to the apiary in the spring of 2019. Data collection took place from October to December 2020; during this season there is a change in bee activity and behavior. In preparation for the winter season, there is a reduction in brood, foraging activities begin to lessen, and honeybee senescence periods are elongated. Diutinus, also known as “winter bees” or “long-lived workers,” are physically and behaviorally different than summer bees; they have excess fat body storages and when brood is absent minimal nursing or provisioning activities are required (Münch & Amdam, 2010; Ricigliano et al., 2018). It is important to note that we conducted this research during the COVID-19 pandemic. Due to campus restrictions, beginning in March 2020, there was limited access and reduced testing for and treatment of potential increase in *Varroa* mite numbers until October 2020. During this time, we lost three of our six colonies. Additionally, of the three remaining colonies, two were deemed unfit to partake in the observational research due to high *Varroa* mite presence, resulting in their immediate treatment with miticide and then subsequent closure for the overwintering months. We collected the data from our only remaining hive, MH-1 (nucleus originated from Massey Honey). Our apiary uses Langstroth hives, two brood boxes deep, and ten deep frames in each box. The external weather data was taken from Fullerton Municipal Airport and the averaged for each week was used to determine the weekly variation.

Powdered Sugar Tests

During our weekly inspections, we assessed the presence and extent of live *Varroa* mite by conducting powdered sugar tests as illustrated in Barlow and Fell (2006) and Lee et al. (2010). We collected samples from brood frames with the most diverse larval stages present. We shook the frames over a plastic bin, then tapped the bottom to prompt all foragers to fly off, leaving us with only the nurse bees in our sample. We then collected 4 fluid ounces, or about 300 individuals, in a meshed-wired topped 12-fluid-ounce mason jar. This is the standard, widely agreed upon sample size (Lee et al., 2010). Any larger ($n > 300$) could be detrimental to smaller colonies (Lee et al., 2010). The goal is to get an adequate sample size without harming the sampling individuals or the colony as a whole. We did not use a rolling alcohol sample to confirm our *Varroa* mite count because the rolling alcohol treatment kills honeybees and we wanted to ensure that we returned every individual to the colony. They were then rolled in 2 tablespoons of powdered sugar and left for 2 minutes undisturbed. We shook the bees over a paper plate until we were sure no more debris or mites are falling out of the jar. We then sprayed the plate with water to reveal the mites and then counted the number present. This sampling method allows for beekeepers to estimate the colony's mite density and determine necessary management strategies (Lee et al., 2010). During this observational research period, MH-1 never had a *Varroa* mite count that exceeded the threshold (10 mites per 300 individuals in the sample or prevalence of 3%).

Varroa **Mite Traps**

To evaluate the frequency of grooming behavior, we placed sticky board mite traps with screen meshes from Dadant on our bottom boards. Each trap was placed after our weekly inspections on Tuesday and was retrieved 3 days later on Friday. For this research, a Skybasic Digital Microscope with a 1000x magnification range and HD resolution of 1920x1080P was used to connect to an iPhone. This device is affordable and easy to use, making it the perfect implement for a beekeeper. For each sticky board trap, the number of mites was counted and then categorized based on age, whether there was damage or not, and the type of damage present if any. The description and identification of grooming damages followed Nganso et al. (2017) Fig. 2 and reconfigured to five categories that could quickly be identified with a readily affordable microscope: (1) Leg(s) Damage(d), (2) Removed Gnathosoma, (3) Hollowed out Shell ("shield"), (4) Chipped or Damaged Shell ("shield"), and (5) Crushed or Removed Scuta. We also referred to images illustrating grooming damage from Hunt et al. (2016). Only *Varroa* mites that had fallen on their backside received a "groomed" or "intact" status because of the position. This is a conservative count of damage due to bee grooming because the legs of individual *Varroa* mite are visible and damage cannot be due to adhesion to the sticky board. As a consequence, all individual *Varroa* mites who fell shell up were classified as "undetermined," because their position made it impossible to discern if it was grooming damage or an attempt to move the mite to a more optimal position would have resulted in non-grooming damage.

Observational Grooming

During weekly inspections, the brood frame determined the best use for a powdered sugar test was also the same frame we used to observe grooming behavior. The decision to use the same frame is based on the location of house bees, individuals most likely to groom their nestmates, and proximity to larval brood (where *Varroa* mite populations are the most frequent). Cini et al. (2020) found that allogrooming events preferably took place in areas on the comb where brood was present in clusters instead of areas like the hive entrance or where dancing took place. The frame (standard Langstroth hive frame sizes are 9 ¾ x 19 inches) selected for the powdered sugar test was removed and placed upright to the side of the brood boxes. A GoPro Hero7 (4k60/1080p240) set at an approximate distance of 1.5-2 feet was set to video the undisturbed frame for 2 minutes. Once the video recordings were completed, the frames were used in the powdered sugar test, as described above.

Behavioral observation coding was conducted using videos in coordination with Noldus Observer XT 15 behavioral observation software. The frequency of grooming behavior was estimated after scoring behavior at each time interval based on our ethogram (see Table 1) of the differing grooming behavior types. We decided to use The Observer (The Observer XT 15) because it allows for the integration and synchronicity of offline collected videos and allows the user to incorporate observational and physiological data (Zimmerman et al., 2009). We defined four main behavioral groups: (1) Body Grooming, (2) Face Grooming, (3) No Grooming, and (4) Cannot Be Seen. The Body Grooming group was defined to include the specific behaviors of Thorax Grooming, Abdominal Grooming, and Other Body Grooming. The Face Grooming group was defined with additional behaviors of Proboscis Grooming, Antennae Grooming, and Other Face Grooming. Identifying the regions being groomed may indicate if honeybees are focusing on high *Varroa* targeted areas (i.e., the abdomen and thorax regions) (Delfinado-Baker et al., 2009). For both the Body Grooming and Face Grooming behavioral groups, we used modifiers. This allowed us to create a hierarchical behavioral scheme. Individual Type (Recipient or Groomer) and Grooming Type (Allogrooming or Autogrooming) helped to discern the type of grooming and in what capacity our observed individual participated. The definition of allogrooming as “an individual touching or rubbing another using their antennae, mandible, or legs to manipulate the individual” follows Land & Seely (2004) and Carr et al. (2020). We have also used the definition of autogrooming or “self-grooming” as “a stationary bee uses its legs and/or mandible to clean their person” taken from Carr et al. (2020).

Figure 3. Grooming behavior. **A.** A hive worker, covered in powdered sugar being groomed by another bee on their thorax region. **B.** A hive worker, covered in powdered sugar being groomed by another bee on their abdominal region. **C.** Two hive workers engaging in proboscis, face grooming.

Individual honeybees were tracked across intervals by taking a screenshot of the first frame for each video using Abode Premiere Pro. All discernable individuals (individuals in the foreground of the video) received a number starting from one. All individuals that appeared to be covered by another bee at the beginning of the video were excluded. A random number generator was used to select 30 individuals per video to be followed with grooming behaviors recorded every 10 seconds for the duration of the video. The event log from Observer XT 15 was exported to Excel to be organized for statistical analysis using SPSS.

Table 1. Ethogram of the European Honeybees (*Apis mellifera*) Grooming Behaviors. The behavioral grooming samples with their Observer XT 15 codes and their modifiers.

Behavioral Groups	Code	Modifiers	Definition
		Body Grooming	
Thorax Grooming	T	*Grooming Type:* Autogrooming Allogrooming *Individual Type:* Recipient Groomer	A bee is using its legs and/or mandible to remove debris from itself or another's thorax region.
Abdominal Grooming	Ab	*Grooming Type:* Autogrooming Allogrooming *Individual Type:* Recipient Groomer	A bee is using its legs and/or mandible to remove debris from itself or another's abdominal region.
"Other" Body Grooming	O	*Grooming Type:* Autogrooming Allogrooming *Individual Type:* Recipient Groomer	A bee is using its legs and/or mandible to remove debris from itself or another's body region.
		Face Grooming	
Proboscis Grooming	P	*Grooming Type:* Autogrooming Allogrooming *Individual Type:* Recipient Groomer	A bee is using its legs and/or mandible to remove debris from its or another's proboscis.
Antennae Grooming	An	*Grooming Type:* Autogrooming Allogrooming *Individual Type:* Recipient Groomer	A bee is using its legs and/or mandible to remove debris from its or another's antennae.
"Other" Face Grooming	t	*Grooming Type:* Autogrooming Allogrooming *Individual Type:* Recipient Groomer	A bee is using its legs and/or mandible to remove debris from itself or another's face.
		No Grooming	
Not Grooming	Ng	N/A	The individual bee is neither participating in the act of remove debris from another bee and/or is not having debris remove from them.
		Cannot Be Seen	
Out of View	u	N/A	The individual bee is either obscured by other bees or has left the view of the camera.

Statistical Analysis

In this study, the proportion of time spent grooming does not range between 0.2-0.8, the established acceptable distribution for analysis using Ordinary Least Squares (OLS) regression (Long, 1997), because when many of our individuals did not participate in body grooming activities, they received a score of zero. Moreover, because of the large number of zeros represented in the data for the proportion of time grooming, the grooming data are also represented as presence/absence. In a binary data form, we were able to run a logistic regression to assess the relationship between relative humidity and *Varroa* mite levels on the presence or absence of grooming. The relationship between the number of live *Varroa* mites and the number of groomed *Varroa* mites, controlling for relative humidity, was assessed using an OLS regression. Measures of association between relevant variables were evaluated using a series of Pearson Produce Correlations and Spearman Rank Coefficients.

CHAPTER 3

RESULTS

Hypothesis 1

Hypothesis 1. If honeybees prioritize reduction of *Varroa* mite through allogrooming, then we expect more time devoted to this form of grooming during periods of lower relative humidity.

Prediction 1.1

Prediction 1.1. During periods of low external humidity, we expect the time invested in body grooming to increase.

The beta values for the logistic regression models using relative humidity and several different measures of *Varroa* mite levels as our predictors for the likelihood of observing grooming were positive (see Table 2). This means that as humidity increases, grooming activities also increase. These findings did not support our prediction 1.1. However, for each model, excluding model 2, relative humidity was a significant predictor for the likelihood of observing grooming ($p < 0.05$). The odds ratio in the reduced model indicates that within each percent that relative humidity increases there is a 1.14-fold increase in the likelihood grooming will be observed. Since there are multiple ways to account for *Varroa* mite infestations within our data and there is a correlation between all variables, by using this approach of multiple models we were able to determine which aspect of the variables was most sensitive to the presence or absence of grooming, leading us to determine which model best predicted the presence or absence of grooming.

Prediction 1.2 & 1.3

Prediction 1.2. As devotion to grooming activities increases, there will be an increase in the number of *Varroa* mites on the gridded sticky boards.

Prediction 1.3. Of these fallen individuals, the majority of mites will show signs of grooming damage.

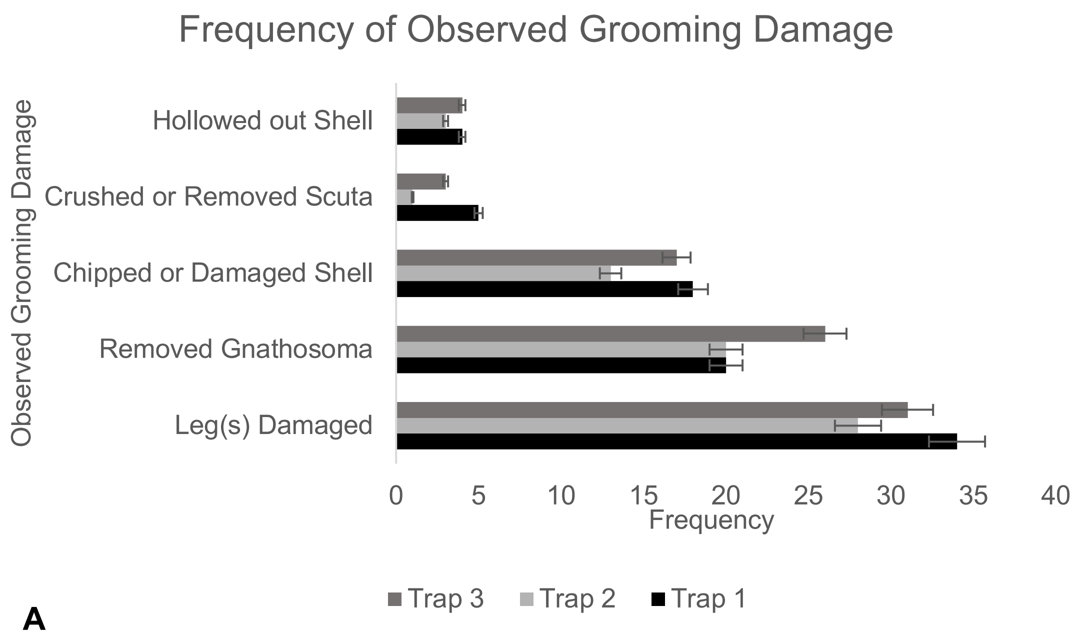
I examined the Pearson Product Moment between the proportion of time spent grooming the thorax region and the number of groomed *Varroa* mites, and the proportion of time spent grooming the abdominal region and the number of groomed *Varroa* mites to test these predictions. The correlations were weak and not significant. Then I ran a Spearman Rank Coefficient, and again the correlations were weak and not significant. However, it is important to note that the output for the

Spearman Rank Coefficient between the proportion of time spent grooming the abdominal region and the number of groomed *Varroa* mites approaches significance ($p < 0.05$). This could be a result of the minimally observed grooming time captured. We only followed 30 individuals in each video. This could have been too small of a sample size, or the use of predetermined time intervals could have placed constraints on collecting more behavioral observations. It is also important to note that the Pearson Product Correlation and Spearman Rank Coefficient between the total proportion of time invested in body grooming and the number of live *Varroa* mites collected during our weekly inspection powdered sugar test were significant ($p < 0.05$, see Table 3 and Table 4). Although the r values are moderate, only 3% and 5% of the variance in proportion of time invested in body grooming is explained by the number of live *Varroa* mites. Interestingly, the correlation analyses show that as the total proportion of time devoted to body grooming increases (or decreases), the number of live *Varroa* mites decreases (increases). An ordinary least squares (OLS) regression analysis indicates there was no significant relationship between the total proportion of time spent grooming and the number of *Varroa* mites removed by grooming.

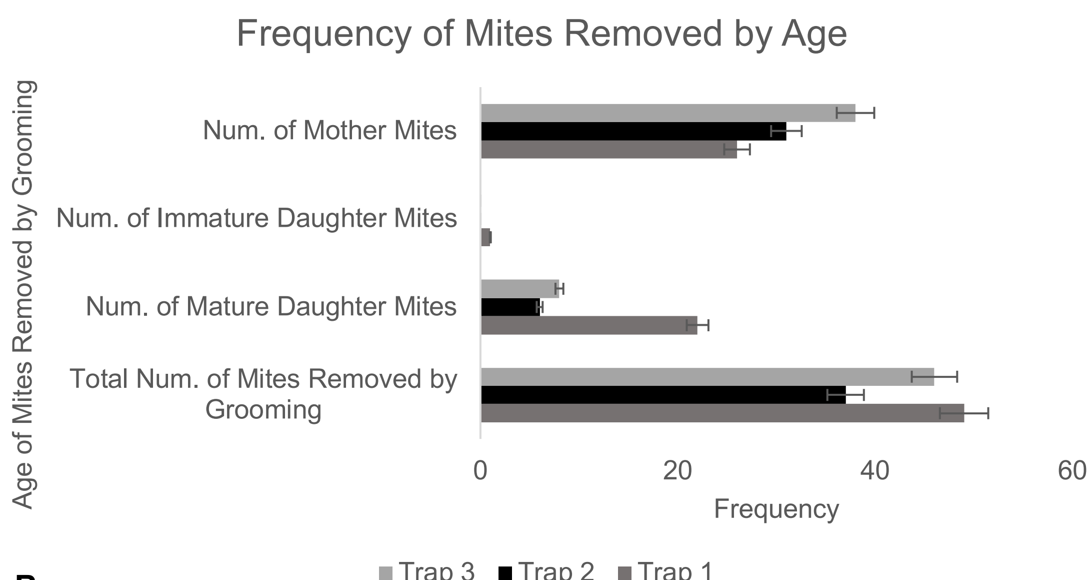
Table 2. Logistic Regression Models. Comparing the effect of different measures of parasitic load and relative humidity on the presence/absence of body grooming.

Variable	β	S.E.	Wald t	Odds Ratio	-2 log-likelihood	*p*-value
Model 1						
Relative Humidity	0.155	0.078	3.932	1.167	71.389	0.047*
Number of dropped *Varroa* mites	-0.048	0.058	0.690	0.953		0.406
Model 2						
Relative Humidity	0.357	0.287	1.550	1.429	71.389	0.213
Number of live *Varroa* mites	0.529	0.637	0.690	1.698		0.406
Model 3						
Relative Humidity	0.155	0.078	3.932	0.953	71.389	0.047*
Number of groomed *Varroa* mites	-0.048	0.058	0.690	1.167		0.406
Reduced Model						
Relative Humidity	0.130	0.062	4.436	1.139	72.083	0.035*

Note: *$p < 0.05$



A



B

Figure 4. **A.** The frequency of the observed honeybee grooming damage collected from the MH-1 *Varroa* mite traps during the 3-week observational period of Nov.- Dec. 2020 (excluding Thanksgiving week when data was not collected). All observed damaged counts came from individuals that were classified as removed by grooming due to their fallen position (on their backs) and all damages were deemed not to be a result of trying to pull themselves off the board. (Error bars at 95% confidence intervals). **B.** The frequency of observed age of *Varroa* mite that were removed by honeybee grooming. Of the number of mites removed, sexual mature “mother” mites were statistically had the highest removal rate of all ages observed in MH-1 hive. Removal of “mother” mites is an important pest management practice for honeybees in order to control reproduction and therefore, reduce the overall mite population. Age of mites were determined based on coloration (mothers are a dark red, mature daughter are a lighter red/pale pink color, and immature daughters are a cloudy white color). (Error bars at 95% confidence intervals).

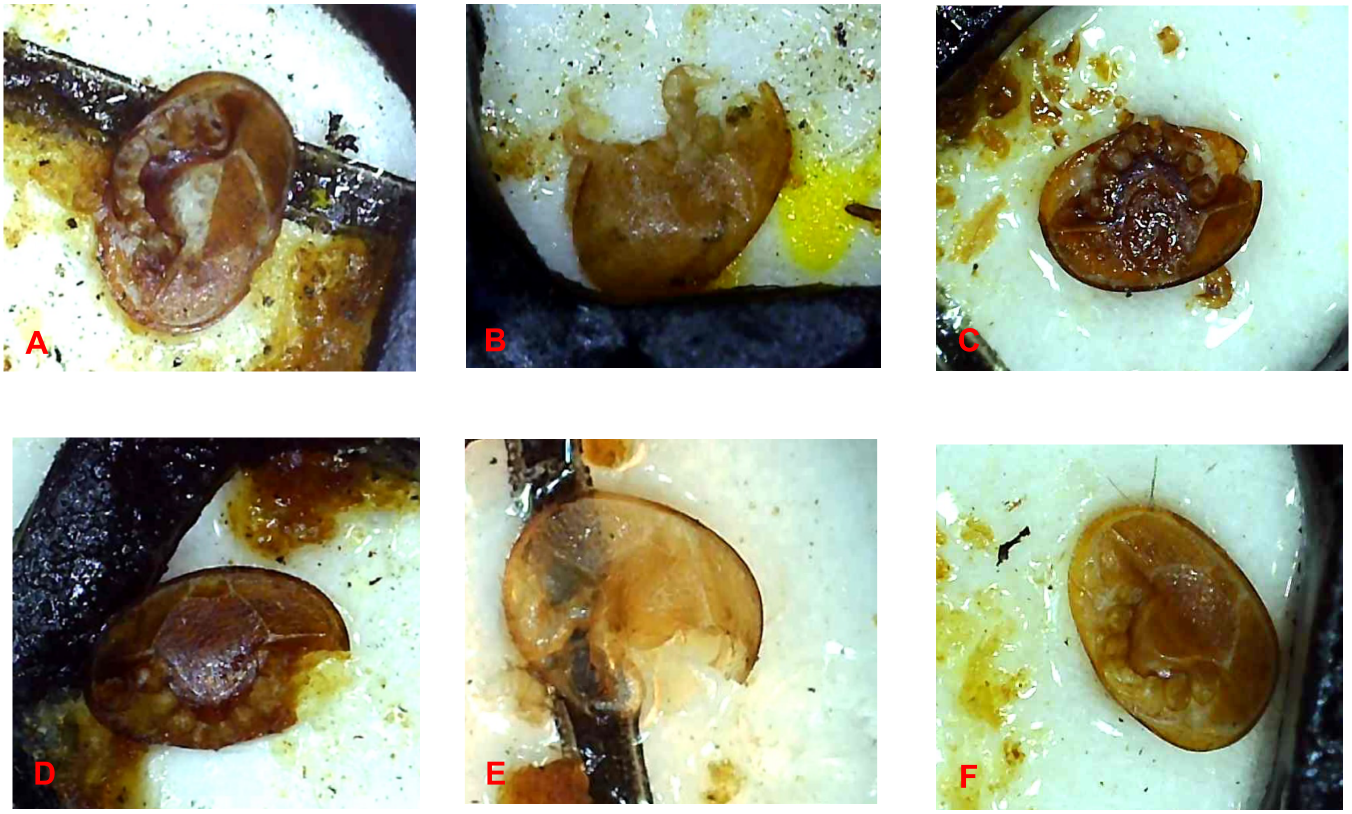


Figure 5. The 5 categories of grooming damages observed. **A.** Leg(s) Damaged, Removed Gnathosoma, Crushed or Removed Scuta. **B.** Chipped or Damaged Shell (other damage could be present but because of the falling position makes it impossible to discern). **C.** Leg(s) Damaged, Removed Gnathosoma, Chipped or Damaged Shell. **D.** Leg(s) Damaged, Removed Gnathosoma, Chipped or Damaged Shell. **E.** Leg(s) Damaged, Hollowed out Shell, Chipped or Damaged Shell. **F.** Leg(s) Damaged, Removed Gnathosoma. Of all the damages observed leg(s) damaged or complete removal were the most frequently observed, followed by the removal of gnathosoma. Both of these damages are also most frequently observed in the grooming literature for *Apis mellifera*.

Based on the sticky board data, it was determined that of the *Varroa* mites that dropped to the bottom, 50% had grooming damage. There is a possibility the number of mites removed through grooming may be much higher due to the conservation approach used for classification. Mites that fell with their shell ("shield") up and therefore with legs obscured, were classified as "undetermined." The observed grooming damage on leg segments or entire leg removal and removal of the gnathosoma were the most common (see Figures 4a & 5).

Hypothesis 2 & Prediction 2

Hypothesis 2. As humidity decreases, we expect the number of *Varroa* mites detected in a powdered sugar test to increase.

Prediction 2. There is a relationship between *Varroa* mite fecundity and relative humidity, and during periods of lower humidity *Varroa* mite fecundity should increase.

We ran a Pearson Product Correlation (see Table 3) and a Spearman Rank Coefficient (see Table 4) between our weekly average relative humidity and the number of live *Varroa* mite counts collected during our weekly inspection powdered sugar test. Our correlation test shows a significantly strong negative correlation between these variables, with 96% and 100% of the variance being accounted for and with a *p*-value significant at the 0.01 level ($p < 0.01$) showing that as external humidity increases (or decreases) live *Varroa* mite populations will decrease (or increase) with it.

Table 3. Pearson Product Correlations. The correlations between the factors of weekly average external humidity and total proportion of time spent grooming, and the number of live *Varroa* mites found during the weekly powdered sugar tests.

	Dependent Variable	*r*	*p*-value
Weekly Average Relative Humidity	Num. of live *Varroa* mites	-0.982**	$p < 0.0001$
Total Proportion of Time Spent Grooming	Num. of live *Varroa* mites	-0.179*	0.046

Note: *$p < 0.05$, **$p < 0.01$ (1-tailed)

Table 4. Spearman Rank Coefficients. The correlations between the factors of weekly average external humidity and total proportion of time spent grooming, and the number of live *Varroa* mites found during the weekly powdered sugar tests.

	Dependent Variable	*r*	*p*-value
Weekly Average Relative Humidity	Num. of live *Varroa* mites	-1.00**	$p < 0.0001$
Total Proportion of Time Spent Grooming	Num. of live *Varroa* mites	-0.219*	0.019

Note: *$p < 0.05$. **$p < 0.01$ (1-tailed)

This correlation shows that as humidity decreases, *Varroa* mite populations increase. This is expected based on the recent studies indicating that *Varroa* mite fecundity decreases with increases in humidity. We typically see a decrease in both the temperature and relative humidity during these winter months and an increase in *Varroa* mite populations. Typically, beekeepers test for *Varroa* mite infestations prior to the overwintering closer to the hives and then after the wintering months are over to determine if miticide treatments are necessary.

CHAPTER 4

DISCUSSION

Hypothesis 1

Prediction 1.1

When biotic and abiotic stress factors, such as *Varroa* mite population increases, are present in the colony, honeybees may address the problems through self-management using social behaviors and immune responses (Li et al., 2018). As hypothesized, if honeybees prioritize reduction of *Varroa* mites through allogrooming, then we expect more time devoted to this form of grooming during periods of lower relative humidity. Additionally, it was predicted that during periods of low external humidity, the time invested in body grooming would increase. The logistic regression models comparing the presence/absence of grooming to relative humidity and various *Varroa* mite presence levels did not support this prediction. The beta value being positive indicates that as humidity increases so does the likelihood grooming behavior will be observed. This may be related to time allocation strategies, whereby trade-offs may be necessary depending on differing stress factors influencing honeybee decision making. During times of high stress or periods when honeybees are experiencing multiple stressors (i.e., low humidity and large pest loads), they may be required to choose between tasks (Li et al., 2018; Southwick & Heldmaier, 1987). The optimum solution for honeybees may be to devote time to maintaining internal humidity levels at the optimal level for brood rearing instead of focusing on parasitic infestations. In the short term, this may take precedence over managing higher parasitic loads. Simpson (1961) states that during periods of abnormally low humidity, for example during winter months, 20% or less may lead to the desiccation of the larva and prevent the rearing of the brood. Interactions between differing biotic and abiotic stressors can increase in severity of their effects on both the health and survivorship of the whole colony (Li et al., 2018). During these periods of time allocation constraints, whereby honeybees must make decisions of where to devote their time and energy, the decisions they make may ultimately have a poor impact on the colony. In Figure 1c, a worker bee is depicted with crinkled wings as it has been infected with deformed wing virus, making it unable to fly. Deformed wing virus spreads through *Varroa* mites, which are also vectors for other viral diseases (Martin, 2001). It can also be seen that the individual worker bee has several *Varroa* mites on its person. When honeybees make decisions to focus energy on tasks like increasing humidity levels to prevent the desiccation of the brood, rather than devoting time to grooming, there may be a prolonged negative effect on the health and strength of the colony by allowing *Varroa* mite populations to increase, possibly increasing in severity and putting the survivorship of the colony at risk.

Furthermore, Doull (1976) found at 50% relative humidity, a large proportion of eggs shriveled, and of the remaining eggs, only 2.9% produced typical larva and no eggs hatched at humidity levels below 50%. For honeybee colonies to reach optimal humidity during winter months where external humidity is relatively low, honeybees must maintain dense clusters to prevent brood desiccation (Simpson, 1961). This means honeybees could be making a decision based on shorter goals (brood rearing) over long-term goals (maintaining lower parasitic loads). Mitchell (2019) states that honeybees in artificial hives need to forage and then desiccate honey ten times more efficiently to obtain a humidity of 4.3 kilopascals (kPa), and this study reports that this humidity level is sufficient to lower *Varroa* mite fecundity. That means that in addition to maintaining an internal hive climate, the colony needs the foragers to work harder to collect the necessary nectar to make that happen.

Currie and Tahmasbi (2008) found that under low humidity, the mites that fell to the bottom of the cage with high-grooming groups were more than twice that of the low-grooming groups ($p < 0.05$). They also found that high-grooming groups' grooming success reached a high of 73% at 77° Fahrenheit and low humidity, over low-grooming groups that only reach a high of 22%. Additionally, Currie and Tahmasbi (2008) found that bee mortality rates are higher in high-grooming lines than in low-grooming lines during periods of lower temperatures and humidity. This means that there could be a biological cost associated with increased grooming behavior during low temperature and humidity periods. It is possible bees that descend from a high-grooming line may intrinsically be inclined to groom in periods of higher parasitic loads than maintain homeostasis of the internal hive environments that would achieve reproductive goals.

Prediction 1.2 & 1.3

It was also predicted as more time is devoted to grooming, the number of *Varroa* mites on the meshed sticky board traps will increase. Therefore, it was predicted that the majority of the fallen individuals will show signs of grooming damage. Prediction 1.2 was not supported; however, there was a strong negative correlation between total proportion of time spent grooming and the number of live *Varroa* mites indicated in the powdered sugar test (see Table 3 and 4). This indicates that as the total proportion of time spent grooming increased, the number of live *Varroa* mites will decrease. Prediction 1.3 was supported by the results. During the researching period the number of mites that dropped to the gridded sticky boards stayed consistent. Additionally, the ratio of groomed *Varroa* mites to the total number of dropped *Varroa* mites were consistent. About 50% of the dropped *Varroa* mites had grooming damage present, with the majority of the damage being consistent with leg(s) damage or removal of gnathosoma (see Figure 4a. & Figure 5). This finding is supported by other studies that observed similar grooming patterns for *A. mellifera* (Nganso et al., 2017).

It is also important to note that, as previously discussed, we could not classify all individuals that fell to the sticky board as “intact” or “groomed” because of how they fell on the board. That means that the number of groomed individuals may be higher than this research study was able to determine. Additionally, of the *Varroa* mites that fell to the bottom, most individuals were sexually mature female mites (see Figure 4b.). There are two reproductive statuses for female mites: mature daughters (individuals that are indicated by their lighter-pinker coloration and have recently been mated) and “mother” mites that have been through at least more than one reproductive cycle (these individuals are indicated by their darker-red coloration). Mother mites can invade a second cell and has been observed to go through eight cycles of reproduction; however, on average two cycles is typical and they can reproduce on average 2 mated female offspring (Fries & Rosenkranz, 1996 and Martin & Kemp, 1997 as stated in Oldroyd, 1999). Being able to determine the age of the mites that were removed by grooming is essential. It means that the honeybees are more often targeting female mites with the capability of reproducing. Removal of reproductive females results in population control.

Hypothesis 2 & Prediction 2

Our second hypothesis is as humidity decrease, we expect the number of *Varroa* mites detected in a powdered sugar test to increase. It was predicted, there is a relationship between *Varroa* mite fecundity and relative humidity, and during periods of lower humidity *Varroa* mite fecundity should increase. The Pearson Product Correlation indicated a strong negative correlation between relative humidity levels and an increase in *Varroa* mite fecundity, supporting prediction 2. This study was conducted during the seasonal change in Southern California. The colony experienced periods of moderate humidity changes, as well as severe changes. The average weekly percent relative humidity ranged between 40-66% with the highest percent relative humidity regions being between 73-93%. During these periods of weekly fluctuations, *Varroa* mite counts also fluctuated. As the average weekly relative humidity and average relative humidity decreased, the number of live *Varroa* mites present, as indicated by the powdered sugar test, increased. Even though honeybees have the capacity to manipulate the internal relative humidity, to keep the relative humidity at optimal levels, individuals are under time allocation constraints. In addition to the need for optimal humidity, they must also manage their internal temperature so that the temperature reaches optimal level for brood. Moreover, humidity and temperature are inversely related. As temperature increases it can displace the moisture in the air, reducing the humidity. Not only are honeybees experiencing trade-offs between biophysical parameters and social immunity responses, but they are experiencing trade-offs between regulating different biophysical parameters such as humidity and temperature (Human et al., 2006). Both parameters require different regulatory mechanisms and activities. Mitchell (2016) found that honeybees that constructed their nests in a tree enclosure, rather than a thin-walled square box, like a Langstroth hive, had higher levels of humidity in the nest and increased their survival of a smaller colonies and lowered the *Varroa* mite breeding success. The ability for honeybees to maintain higher humidity levels correlates directly to the ability to mitigate *Varroa* mites' reproductive success.

Conclusion

The ability to use weekly hive inspections and incorporate behavioral observation data has been informative regarding individual apiary environmental constraints and in conducting research that best assists each apiary. This research has also indicated a specific niche that has not been filled in honeybee research. Much of the research done regarding *Varroa* mites and honeybee grooming is done in sterile lab settings where they are controlling for many of the additional factors (i.e., temperature, humidity, and the number of mites added artificially). However, this research has taught us that these biotic and abiotic factors are much more complex and correlated than initially thought. As Figure 2 indicates, there are many factors that affect the overall health and strength of the colony. Moreover, all of these factors are interconnected. Many of these factors are human-made and require a form of human management to understand to what degree human activities play in honeybee colony declines.

As indicated in Figure 2, the model shows the flow of arrows of the complex interconnectedness between each of these factors and honeybee colony health. Beekeeper practices are influenced by so many external factors and in turn directly affects honeybee colony health. Beekeepers must adjust their practices as they are being informed by their local environment. Skilled apiarists are able to recognize different pollination situations and adjust their practices that will affect the future of the hive (Phillips, 2014). As our results indicate, there is a correlation between environmental conditions, parasitic load, and honeybee behavior that all ultimately affect the colony health and strength. Each local environment is different from one another and is affected by different environmental conditions (e.g., temperature, humidity, and wind), including having their own independent seasonal changes. On an individual basis, beekeepers must determine the best management strategies for their own colonies based on their local environment. For example, regions that don't experience a true winter season, like in the tropical zones, may not experience the same seasonal and behavioral changes that a beekeeper in Europe might. Even within the United States, there is high variability amongst the local seasonal changes. In Southern California we don’t experience the same temperature drops that may be experienced in other states, but advanced urbanization limits the local food sources, causing additional stressors on colonies reared here. Additionally, there is a difference in resource availability. Moreover, traditional beekeepers in areas, like Tanzania or Brazil, may not have the same equipment as those typically used in the United States, such as the Langstroth hive boxes. However, this may play to the advantage of these local groups as Mitchell (2016) indicates that tree enclosures imply higher levels of humidity in the nest, and therefore, increased survivorship of the colonies. Tree enclosures allow for honeybees to engage in heat conserving behavior rather than humidity management. Mitchell (2016) implies tree nests and their thermal properties could solve problems like *Varroa* mites and humidity management in apiculture.

This research shows that external relative humidity plays a crucial role in honeybee grooming behaviors and the promotion or attenuation of *Varroa* mite fecundity. More research is needed to gain a considerable understanding of honeybee grooming in real-time and what factors contribute to the efficiency of honeybee activities. It shows that we have yet to grasp the role that life history plasticity plays in the promotion or attenuation of *Varroa* mites. As indicated by our results, as relative humidity increases grooming also increases, which means that our honeybee colony was not grooming during periods of low humidity like we thought. This could mean our honeybees are conducting a risk aversion strategy to maintain internal hive climates or other tasks not related to grooming while allowing *Varroa* populations to increase. Honeybees may be participating in cost-analysis to accomplish short-term goals while allowing their longer-term goals of reducing parasitic loads to be placed on hold. There is a possible link between grooming and task management with the reduction of the hive’s population. If the population begins to decrease before the overwintering months, the colony may not have enough individuals to devote equally to all tasks. Therefore, some tasks, like grooming, may not have an adequate number of honeybees to participate in this activity to deal with the *Varroa* mite infestation.

Due to the shortened data collecting period, caused by COVID-19, the amount of data collected for certain aspects of this research was not sufficient for specific analyses. Nevertheless, this type of research could help develop better methods that beekeepers could employ at their apiaries, making research more accessible to them and their individual needs based on their local environmental conditions. Moving forward, scientific methodology and beekeeping experiences must come together to find the causality between these various biotic and abiotic factors, in addition to how they pertain to CCD. Without a clear understanding of the interconnectedness between humans and nature, we will not be able to think about the environment using a more-than-human approach. Moreover, we will not be able to solve our agricultural issues and finally put limits to our destructive agricultural practices. We will continue to see the result of more losses to honeybees and other pollinator populations. Human cooperation will be the only way we solve the *Varroa* infestations, mitigate pesticide damage, and better promote honeybees' adaptive behavioral responses to these stressors in the environment. Diverse thinking and knowledge centers are critical in understanding and solving environmental problems (Maderson & Wayne-Jones, 2016).

Limitations

Unfortunately, due to the COVID-19 pandemic, our intended research periods were cut short by months. We were only able to collect a small sample a few weeks before the closure of the apiary for the overwintering months. As stressed previously, leading up to the overwintering months, we expected *Varroa* mite populations to increase, but the climate was also in their favor. We would have preferred to sample over the different months and get a clearer understanding of the seasonal changes and honeybee behavior associated with those changes. Additionally, due to the state-wide shut down, our apiary and hives received minimal management, which resulted in the loss of three of our six colonies, and high *Varroa* mite infestation events. This left us with one hive to conduct this research study. For future research, we would like to make a cross-comparison of our other hives.

The nuclei from the other two hives absent from the study are from Randy Oliver, while our study focused on a nucleus that originated from Massey Honey. We know from preliminary data from the previous year that there are some differences between the Randy Oliver and Massey Honey nuclei and their *Varroa* mite infestation rates.

Additionally, due to the short sampling period, we had to select a smaller sample of individuals to observe and chose an interval sampling strategy. Moreover, though our data shows that the honeybees appeared not involved in a significant amount of grooming, grooming events were happening between interval periods that we were not counted or individuals not included as part of the sample were observed grooming during the videos. We may need to change our observational sampling technique in the future to a continuous sampling method to garner a more comprehensive understanding of the total proportion of time spent on body grooming.

Future Research

The goal for the apiary in the coming year is to establish internal hive sensors. This research suggests that external humidity plays a crucial role in honeybee grooming activities and *Varroa* mite fecundity. However, we would like to see how honeybees are adapting to internal climate patterns. Are honeybees actively changing their internal climates? During which periods of the day do we see a difference in external and internal humidity? Are honeybees' grooming periods associated with internal humidity and temperature changes? Observing internal climate changes and *Varroa* mite presence and using a continuous behavioral grooming sampling method may give us an even clearer picture of the relationship between these factors and Colony Collapse Disorder.

APPENDIX A

WEEKLY INSPECTION FORM

Hive Name: _______________

Hive Inspection Type: (*select one*)

- o Full Inspection (Looking at all Brood Boxes + *Varroa* Mite Check)
- o Quick Inspection (Looking at one Brood Box for key characteristics)
- o No Inspection was done today

Date & Time: ________________

Weather (describe temperature, humidity, and wind)

Temper of the Hive (*select one*)

- o 0: Baseline: absence of behavior
- o 1: Mellow/calm: bees flying slowly, guard bees fly by slowly and then leave. Typical curious behavior.
- o 2: Slightly irritated: Guard bees emerge at time hive is opened, bees flying fast, but do not cluster around beekeeper's head, bees do not try to sting through gloves.
- o 3: Irritated/Increasingly defensive: Many guard bees emerge when hive is opened, bees flying fast, cluster around head, hitting veil, press their abdomens into veil in an attempt to sting, bees hit gloves and attempt to sting.
- o 4: Localized Defensiveness: Guard bees are flying into the veil of the beekeeper, creating a "pinging" sound and guard bees continue to follow the beekeeper throughout the apiary even once the hive has been closed up.

Lethargic Behavior Presence (*pick all that apply*)

- ☐ Abdomen dragging
- ☐ Stopped/standing still
- ☐ Upside down (on ventral surface and attempting to preform righting reflex)
- ☐ Curled up (laying on side/hunched over)

Laying Pattern (*select one*)

- o 1: honey present, no pollen, no brood
- o 2: honey in corners and end of frames, no pollen, low amount of brood of various size
- o 3: honey in corners and end of frames, no pollen, spotty brood patch of various sizes
- o 4: honey in corners and end of frames, no pollen, solid brood patch
- o 5: honey in corners and end of frames, pollen arc, solid brood patch

Grooming Behavior Presence (*pick all that apply*)

- ☐ Proboscis grooming
- ☐ Body grooming
- ☐ Antennae grooming
- ☐ No grooming behavior was observed

<u>Hive Configuration</u>

Number of Honey Supers: _______________

Number of Brood Boxes: ________________

Feeder (i.e., syrup)

- ○ Yes
- ○ No

Entrance Block Present

- ○ Yes
- ○ No

Mouse Guard Present

- ○ Yes
- ○ No

<u>Problems</u>

Varroa Mite Check/Methods (*pick all that apply*)

- ☐ Powdered Sugar
- ☐ Alcohol
- ☐ Not performed

Number of Bees in Sample (in oz) (which equals 300 individuals): ______________

Number of *Varroa* Mites Present: _________________

Pests Present: ___________________

Brood Disease Present/Which type:

- ○ AFB
- ○ EFB
- ○ Chalkbrood
- ○ Other: _________________

Adult Diseases Present

- ○ Nosema
- ○ Acarine
- ○ Viruses
- ○ Other: _________________

<u>Actions & Tasks</u>

Pollen Patty Added

- Yes
- No

Pollen Patty Amount Added

- 1/8 lb
- 1/4 lb
- 1/2 lb
- None

Drone Frame Scrapped

- Yes
- No

Treatments

- Apiguard
- Oxalic Acid
- Formic Acid
- No treatment was added
- Other

To Do's: ____

____

Super & Frame Statues

Number of Bee Seams Present in BB Lower: ________________________

Number of Bee Seams Present in BB Upper: ________________________

Queen Observed

- Yes
- No
- Yes, but she is not marked

Was there evidence of Queen-lessness?

- She was not seen and eggs were not seen
- Both

Were eggs present?

- Yes
- No

Where were the eggs seen?

- Brood Box (Lower)

- Brood Box (Upper)
- Both Brood Boxes Lower & Upper

Was larva present?

- Yes
- No

Where was the larva seen?

- Brood Box (Lower)
- Brood Box (Upper)
- Both Brood Boxes Lower & Upper

Was there sealed brood (does not include Drones)?

- Yes
- No

Where was the sealed brood seen?

- Brood Box (Lower)
- Brood Box (Upper)
- Both Brood Boxes Lower & Upper

Were there Sealed Brood on the drone frame?

- Yes
- No

Were eggs found on drone frame?

- Yes
- No

Was larva found on drone frame?

- Yes
- No

Was sealed drone brood found outside of the drone frame?

- Yes
- No

Was honey Present

- Yes
- No

Was the honey capped?

- ○ Yes
- ○ No

Honey quantity observed

- ○ Low amount (very little found on frames)
- ○ Medium Amount
- ○ High amount (frame is covered)

Was pollen present?

- ○ Yes
- ○ No

Pollen Quantity Observed

- ○ Low amount (very little found on frames)
- ○ Medium amount
- ○ High amount (frame has a representative band of pollen)

Was there drawn comb?

- ○ Yes
- ○ No

Were there frames with foundation?

- ○ Yes
- ○ No

Additional Notes: ____

____

APPENDIX B

VARROA MITE TRAP FORM

Hive Name: ___________________

Date the trap was placed: _____________________

Date the trap was removed: _______________________

Quadrant Letter: _____________________

Quadrant Number: _________________________

How many *Varroa* mites are present? ________________________

How many of the mites are mature daughters? ____________________

How many of the mites are immature daughters? ____________________

How many of the mites are mothers? _____________________

How many *Varroa* mites are intact? _____________________

How many of the *Varroa* mites are undetermined status (cannot tell based on placement)?

____

How many *Varroa* mites are mangled (i.e., been removed by grooming)? _____________________

Indicators the *Varroa* mite was removed by grooming: (*pick all that apply*)

- ☐ Damaged legs
- ☐ Damaged shell
- ☐ Crushed scuta (underside)
- ☐ Damaged gnathosoma (mouth part)
- ☐ Hollowed out shell

How many of the mangled mites are mature daughters? ________________

How many of the mangled mites are immature daughters? _________________

How many of the mangled mites are mothers? ____________________

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