HONEY BEE HEALTH AND THE NC STATE APICULTURE PROGRAM

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Background to honey bees, beekeeping, and colony losses

Honey bees (*Apis mellifera* L.) are the primary managed insect pollinators in ~100 crops in commercial production agriculture in North Carolina and the United States. It is estimated that pollination services account for ~$20B every year in added economic productivity, such that roughly a third of our diet depends either directly or indirectly on bee pollination (Aizen et al., 2009). While native bees are also critically important pollinators in these same systems and contribute to this economic impact, they are less amenable to commercial production agriculture because most cannot be manipulated and semi-domesticated like honey bees and thus transported in and out of crops to augment the local pollinator community as required by most growers.

Honey bee colonies are comprised of ~30,000-50,000 individuals, the vast majority of which are sterile female workers that are all daughters of a single reproductive queen. The colony therefore acts as a collaborative, functional family unit where all members perform tasks for the betterment of the group. While the sole function of the queen is to lay eggs, the workers perform all other duties of the colony, including raising of the young (brood), constructing the wax substrate that forms the nest, defending the colony from intruders, and (importantly) venturing from the hive to collect floral food resources (pollen and nectar). The forager bees, therefore, perform the pollination ecosystem service by visiting flowers to collect their food.

Humans have semi-domesticated honey bees dating back several millennia by providing them nesting boxes (beehives). Initially maintained solely for the purpose of honey production, honey bee colonies have since become intimately integrated into agriculture for the purposes of pollination. As the primary managed pollinator, growers typically enter into contractual agreements with beekeepers to have them place their hives in and around a target crop during bloom. As the scale of agriculture has grown over the last few decades, so has the scale of apiculture such that currently the vast majority (~90%) of managed beehives in the United States are owned by a small percentage of the beekeepers (~10%) that physically transport their colonies all across the country to provide pollination services (Lee et al., 2015).

The increased management, scale, and scope of commercial pollination by beekeepers have resulted in increasing challenges to the apiculture industry. As such, it is now much more difficult for beekeepers to keep their colonies healthy and thriving. This is particularly true during the winter as colonies have only their honey stores to survive the foraging dearth, thus many colonies die over winter. Monitoring the honey bee population has gained increased priority for quantifying annual losses, which have been shown to be much higher than what beekeepers self-identify as acceptable and is therefore unsustainable (Figure 1). Determining the causal factors behind the increase in colony mortality, and identifying means to mitigate them, have therefore become top priorities for agricultural, research, and regulatory agencies.

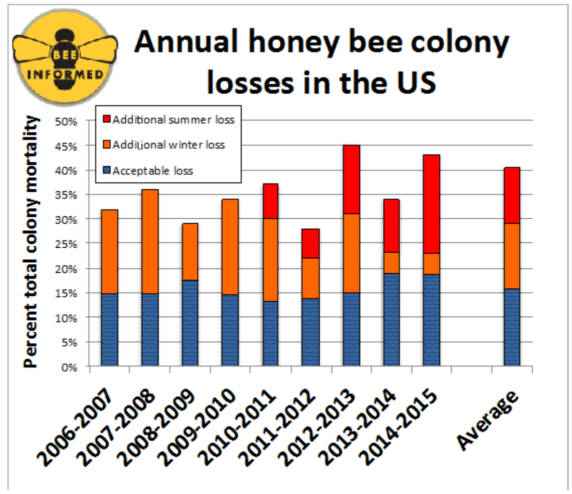


Figure 1. Annual surveys by the Bee Informed Partnership (BIP) have demonstrated that the winter losses (orange) and summer losses (red) of managed honey bee colonies in the US have far exceeded the self-identified 'acceptable' losses (blue) that beekeepers can sustain.

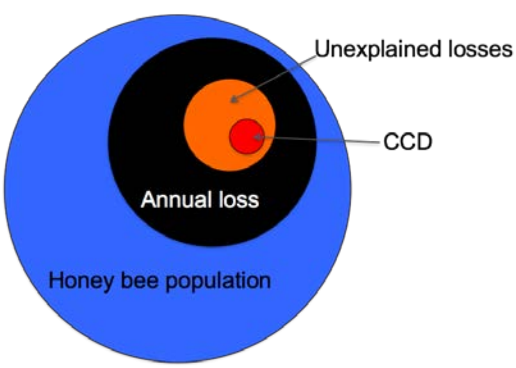


Figure 2. Only a small fraction (~8%) of colony losses are from Colony Collapse Disorder, thus focusing on all problems that beekeepers are facing will be critical to maximize colony health and productivity.

Factors affecting honey bee health

The underlying causes of honey bee colony ill-health and therefore mortality is generally accepted as a complicated interaction of multiple factors rather than any one singular cause. Increased colony mortality is not unique to large-scale commercial pollinators, as the same problems can befall backyard "hobbyist" beekeepers as well (Lee et al., 2015). Nonetheless, untangling the web of threats to honey bee health has been difficult because they are so numerous. The increased media attention to the problem, however, often equates all factors of honey bee mortality with 'Colony Collapse Disorder' or CCD—a relatively new yet still unidentified phenomenon that follows a very specific set of symptoms (vanEngelsdorp et al., 2009). However, of the approximately one-third of the US honey bee population that dies off every year, only a quarter of those derived from unexplained reasons and only a fraction of those are consistent with the symptoms of CCD (Figure 2). As such, keeping the focus on the known factors that affect colony health will be critical in any effort to maintain a stable honey bee population.

Parasites, pathogens, and pests

Like all domesticated animals, honey bees are hosts to a wide variety of disease-causing agents that can result in poor health and mortality. Such parasites can be exacerbated through beekeeper management, such as close consolidation of hives, equipment and frame sharing, and lack of proper control strategies. Covered here is only an abbreviated summary of some of the major disease agents of honey bees, but see the Apiculture Mid-Atlantic Pest Management Strategic Plan (PMSP) for a more comprehensive list.

Varroa mites. The parasitic mite *Varroa destructor* is generally considered the main concern for bee health worldwide, and it is the top-ranked disease agent in BIP surveys (Figure 3). This ectoparasite feeds on the hemolymph of adults and developing bees, prompting malformations, undermining colony performance, and eventually resulting in colony death. The original host of *V. destructor* is the Eastern honey bee (*Apis cerana*), but it is believed that the mite host-switched to *A. mellifera* in the first half of last century in regions where both species of bees were managed (Rosenkranz et al., 2010).

Importantly, recent research has highlighted that varroa mites are important vectors of numerous honey bee viruses. In doing so, the mites can compromise the immune systems of their hosts that enable the viral pathogens, which are normally relatively benign, to flourish and manifest in disease. The nearly ubiquitous Deformed Wing Virus (DWV) is particularly problematic and is often associated with heavy varroa infestations, as it is cryptically symptomatic (unlike many of the other viruses) by causing severely wrinkled wings in infected workers that causes them to be less productive for the colony and die prematurely. Other viruses, such as Black Queen Cell Virus (BQCV), Acute Bee Paralysis Virus (ABPV), and Chronic Bee Paralysis Virus (CBPV) are less obvious in their symptoms but have been highly suspected in colony losses and poor health.

	2009	2010	2011	2012	2013	2014	Avg.
Starvation	37%	59%	39%	31%	30%	36%	37%
Weak in fall	11%	24%	34%	34%	32%	33%	28%
Weather	16%	52%	31%	10%	18%	46%	26%
Queen failure	20%	17%	24%	32%	26%	19%	24%
Varroa	15%	21%	20%	17%	23%	17%	20%
Nosema	7%	7%	12%	6%	6%	5%	7%
CCD	6%	8%	7%	9%	11%	7%	8%
Pesticides	4%	5%	5%	7%	8%	7%	6%
SHB			4%	4%	6%	5%	5%
No. beekeepers	571	1587	2682	2887	4681	4903	
Reference	vanEngelsdorp (2010). *JAR*	vanEngelsdorp (2011). *JAR*	vanEngelsdorp (2012). *JAR*	Spleen et al. (2012). *JAR*	Steinhauer et al. (2014). *JAR*	Lee et al. (2015). *Apidologie*	

Figure 3. A compilation of the six most recent annual surveys by the Bee Informed Partnership (BIP) for the self-identified explanations of why beekeepers reported the underlying cause of colony mortality. Percentages do not sum to 100% because mortality rates are averaged across years and because factors are not mutually exclusive.

Pathogens. The number of microorganisms that affect bee health are as numerous as they are diverse. Historically, the most noxious pathogen is the spore-forming bacterium *Paenibacillus larvae* that causes American foulbrood disease or AFB (so named because of the putrid smell produced by the larvae that succumb to infection). Because the spores are incredibly persistent, infectious, and long-lived, AFB outbreaks can occur readily and rapidly. Other economically important pathogens include the fungal disease 'chalkbrood' (*Ascophera apis*) and European foulbrood (*Melissococcus plutonius*), both of which tend to be secondary infections as a consequence of stress rather than primary means of colony mortality.

Nosema. Nosema disease, historically caused by the microsporidian *Nosema apis*, is a parasitic infection of the adult honey bee midgut. Recent evidence suggests that North American honey bees are infected with a different species of, *N. ceranae*, which is thought to be recently introduced to the U.S. Described in the Asian honey bee, *Apis cerana*, in the mid-1990's, *N. ceranae* has been shown to be widespread in European and U.S. honey bee populations. This species is reported to be more virulent than its endemic sister species, causing bee paralysis and rapid declines in colony population. Infected colonies are less productive and more likely to die over the winter, and it is the second most problematic disease agent behind the varroa-virus complex (Figure 3).

Hive pests. Small hive beetles (SHB), *Aethina tumida*, were introduced from South Africa and first found in South Carolina and Florida in 1998. These hive pests are now widespread in the U.S. Hive beetles attack even strong honey bee colonies, where the larvae consume colony resources and defecate in honey, leaving behind a sticky, fermenting, and unusable mess. Heavily infested hives are sometimes abandoned by the bees, and beekeepers have no recourse but to replace damaged hive equipment. Another opportunistic pest is the greater wax moth, *Galleria mellonella*, that damages the wax comb of colonies only once the hive is abandoned or, more often, in stored equipment. Neither hive pest, however, is a major cause of colony mortality (Figure 3).

Pesticides and environmental contaminants

The interface between honey bees and agricultural chemicals has always been at odds because insecticides that are adept at killing pest insects are typically equally effective at killing beneficial arthropods such as pollinators. The opposing priorities for growers makes it particularly challenging for managed and non-managed bee populations in agricultural settings. Information about the relative toxicity of different pesticides to honey bees can be found in Chapter V of the NC Agricultural Chemicals Manual https://content.ces.ncsu.edu/north-carolina-agricultural-chemicals-manual/insect-control.

Because foragers from a single honey bee colony can range upwards of 4 miles from their hive, a single colony can be exposed to anything in their environment within the adjacent 50 square miles. Moreover, because bees are central-place foragers and return to their hive with their collected food items, a honey bee colony can be a biomonitor of the surrounding landscape. Any compounds, both natural or human-made, can therefore be sequestered in the hive matrix (the wax comb or honey stores). Studies have shown that managed colonies are effective reservoirs of agrochemicals, where 121 different pesticides and their metabolites were found in a broad survey of commercial honey bee colonies (Mullin et al., 2010). Nearly ubiquitous were beekeeper-used acaricides to control varroa mites, thus understanding how management practices interact with environmental compounds is of critical importance to honey bee health.

Lack of nutrition and habitat loss

Much attention has been paid to the landscapes in which managed honey bee colonies reside. Specifically, as large-acreage monocultures have become more commonplace in agriculture, their effects on the forage availability on pollinators has been brought into question. At issue is how different plant sources provide distinctly different pollen and nectar that can vary widely in their nutritional value. A balanced diet is important to all animals to maintain proper growth, development, and immune function, and as such certain agricultural landscapes monopolized by nutritionally depauperate crops can have significant consequences for colony health. Indeed, the top beekeeper-identified cause for colony mortality (Figure 3) is starvation, which is an indication of a lack of available forage or proper nutrition of those resources. Poor resource availability can occur outside of agroecosystems as well and is not merely a function of monocultured crops. Habitat loss, and the paucity of adequate bee-friendly habitat, can also significantly contribute to declines in managed pollinators.

Genetic diversity of managed populations

Breeding practices in the apiculture industry can lead to potential genetic bottlenecks in the managed honey bee population. Of the ~2.7 million beehives in the US, a significant proportion of the genetic stock derives from a relatively small subset of 'queen producers' located primarily in CA, HI, or GA; the majority of the 1 million queens produced and sold every year in the industry derive from an estimated 600 queen mothers (Delaney et al., 2009), suggesting that there may be insufficient genetic diversity at the population level to respond to different environmental challenges. Despite the putative bottlenecks within the industry, there appears to be sufficient genetic diversity in the US population (Harpur et al., 2012) although it remains unclear how such genetic admixture might affect bee health.

Associated with genetic factors in the honey bee population is that 'queen failure' is a top management concern for beekeepers (Figure 3). At issue is the reduced longevity of queens, premature queen replacement or supersedure, failed queen replacement, and early failure in egg laying. Whereas queens frequently lived 3-4 years in past decades, today beekeepers find it rare for queens to live beyond a single season. Because queens are the sole source of genetics inside the hive, understanding the genetic background of queens (and the drones with which they mate) is critical to determine the means by which honey bees respond to different environments.

Research and resources at NC State

The NC State Apiculture Program is involved in numerous investigations and projects to address many problems that face the apiculture industry. We have taken a multi-faceted approach to address bee health in an effort to mitigate the underlying factors.

Queen mating behavior and genetic diversity

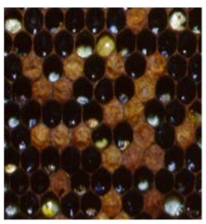
Because queens are so critical to colony productivity and health, our research focuses on understanding the mating success of queens in an effort to improve colony health. Specifically, we have investigated the advantages of queen polyandry (mating with multiple males) and its consequences on colony phenotype. We have shown that queen multiple mating, and the resultant intracolony genetic diversity that it confers, plays a central role in colony survival, productivity, and health. We also investigate the regulation of honey bee reproduction by elucidating the genomic and physiological changes in queen bees during mating. We have used techniques such as instrumental insemination, gene-expression, classical behavioral observation, and GC-MS to determine how virgin queens (who are receptive to mating) transition to laying queens (who never mate again in their entire lifetimes).

Molecular and pollination ecology

Our collaborative work on pollinator diversity and efficiency in commercial crop production has developed interdisciplinary methods that integrate risk assessment into pollination management strategies. In doing so, we have investigated how honey bees fit into the greater pollination communities of various cropping systems to address pollination demands. We are also testing the impact of various pollinator-friendly plantings on local pollinator populations using a molecular ecology approach. Moreover, urbanization is one of the greatest forces of environmental change affecting the world today and is seen as a driving force of climate change. By measuring the disease presence and physiology of different pollinators in urban, suburban, agricultural, and natural ecosystems, we have provided insights into the relative effects of environment on several important factors that influence their productivity and health. Finally, we are also investigating the status of feral honey bee populations in the US by measuring non-managed honey bee populations in the United States to determine its genetic composition and to finally address whether feral populations offer insights into improving the managed honey bee population.

Oxidative stress and social immunity

Stress resistance is an important trait for honey bee health and performance that needs to be evaluated and selected to sustain honey bees for an increasingly managed, industrial application. The oxidative damage and its consequences are a function of the exposure and the internal defenses against oxidative stress. Our research has attempted to quantify variability in oxidative stress and lifespan within and among honey bee colonies. Similarly, we also examine the social context of stress- and parasite-resistance mechanisms to better understand the evolution of physiological and behavioral immunity in social systems. These group-level defenses—known as social immunity—emerge from collective behaviors of individuals that arise to resist persistence of infection at the colony level. Our research takes an integrative approach to understand individual immunity, genetic diversity, and social immune defenses on group-level fitness.



Parasite and pathogen IPM

Integrated pest management (IPM) is a central tenet of agricultural and apicultural practice. Implementing an IPM strategy for economically important pests, most notably varroa mites (*Varroa destructor*), is therefore critical for beekeeping to be sustainable. We have investigated the Russian stock for their efficacy at lowering mite levels across different habitats in North Carolina and management practices. Moreover, as participating members of the Bee Informed Partnership, we have been working with beekeepers and BIP tech-transfer teams to quantify baseline levels of economically important viruses to determine their effects on colony health.

Queen reproductive quality

Because “queen failure” is among the top management concerns of beekeepers (Figure 3), we investigate honey bee queens by looking at their physical quality, insemination success, and mating numbers in an effort to bolster queen reproductive potential and longevity. We also manipulate queen quality by rearing older worker larvae, which has provided insights into the link between queen quality and colony productivity, mating success, and larval development. Our integrative research program is also identifying causative genetic factors affecting honey bee queen quality and production in an effort to increase both. Our research has demonstrated marked variation among honey bee strains for traits associated with queen quality and production.

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