**Comparing colony productivity and pathogen infection between California-bred and New England-bred honeybees (*Apis mellifera)***



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August 2016

**ABSTRACT**

Honeybees (Apis mellifera) are global pollinators that have been on the decline due to various environmental and pathogen-related factors. This decline negatively impacts various ecosystems as well as agriculture. In this study, we tested the hypothesis that locally-bred northern queen bees produce offspring that are more fit in terms of colony productivity and pathogen resistance in their native region than bees brought in from a much different climate. In particular, we compared queens grafted in Vermont with queens brought in from one of the largest queen breeders in California. In terms of productivity, we demonstrated that the local colonies grew significantly more in total colony biomass than those of the Californian bees during specific time steps. This indicated that the locally-bred *A. mellifera* had a stronger foraging tendency. Despite this, we did not find a notable correlation with queen bee origin and mass of pollen collected. We also found that over five time steps, the locally-raised bees reared significantly more brood than the Californian bees, suggesting that the northern queens were more prolific and/or had higher ovary activation than the Californian queens. In assessing resistance to parasites and pathogens, we found no significant difference in levels of *Varroa destructor* over time. However, levels of *Nosema sp*. (a microsporidian) in both the local and CA colony groups differed through three time steps. From T1 to T2, *Nosema* spore loads significantly decreased in the local bees, while spore loads in the CA colonies increased. **The third measurement showed that …** This finding strongly suggests that local bees are more resistant to this strain of *Nosema* and perhaps have heightened immune gene expression. Using PCR to evaluate the percent prevalence of DWV, BQCV, and IAPV (three common RNA viruses in *A. mellifera*), we found that the percent of colony infection before and after requeening with the experimental queens was relatively unchanged between the two groups. Overall, this study supported our null hypothesis that the offspring of locally raised queens from the Northeast are equally adapted for survival and productivity in northern regions as compared to queens and worker bees from California, a climatically contrasting environment. These results present evidence that queen bee origin and environmental adaptions relating to *A. mellifera* fitness could be tied to colony survivability.

**INTRODUCTION**

The honeybee (*Apis mellifera*) is responsible for the pollination of many different crops necessary for agricultural sustainability. Some of these commercial crops include almonds, oranges, and apples. In 2012, the U.S. pollination industry alone is responsible $15 billion a year in agricultural revenue. (1). However, these ever-important pollinators have been in steady decline for the past several decades, and with the rise of Colony Collapse Disorder (CCD) in 2006, colony losses have been even greater for commercial and hobbyist beekeepers. In 2006, winter losses were unusually high, with beekeepers reporting losses of 30-90% (2). More recently in 2015, the nation’s annual colony losses were 42.1% (3). There has been much debate as to the causation of this severe collapse in honeybee populations. RNA viruses, parasites, bacterial and fungal infections, pesticides, and migratory stress have all been thought to contribute to *Apis mellifera*‘s decline as well as CCD (4,5). However, colony fitness, as determined by local environmental adaptations and queen bee genotypes, may also be a contributing factor to the survivability and productivity of honeybee colonies throughout different geographical regions (13).

During their mating flights, virgin queens usually mate with 7-17 drones, helping to acquire genetic diversity and overall survivorship (6, 9). Upon its return to the hive, the fertile queen’s ovaries become activated, laying up to two thousand eggs a day (8). Because the queen becomes the only reproductive female in the colony, the genotypes of queen bees (and the drones they mate with) dictate the genotype of every worker bee in the colony (9). Consequentially, queen bee genomes are partly responsible for expression of behavioral and physiological traits in the worker bees.

Epigenetic theories suggest that species can inherit phenotypic alterations influenced by specific environmental interactions over extended periods of time (14). In accordance with these concepts, beekeepers and researchers are finding that local honeybee stock may have certain fitness advantages over imported bees that have been bred in climatically and ecologically different environments. Such advantages may be related to nectar and pollen foraging behavior influenced by daytime temperatures and relative humidity (10). Other fitness factors may be guided by resistance to pathogen strains that might be less prevalent in the imported animals’ original region (11). This concept of locally adapted strains of *A. mellifera* is both biologically and agriculturally relevant because many beekeepers purchase queens from California and other regions of warmer climates, where queen cells can be produced almost year round.

It is relatively common knowledge in the beekeeping industry that certain subspecies of *A. mellifera* have different behavioral and morphological characteristics. *Apis mellifera carnica* and *Apis mellifera ligustica,* for example*,* are two of the most common domestic honeybee strains. *Apis mellifera carnica* are known for thriving in colder climates, having smaller populations, and being prone to swarming (12). In comparison, *Apis mellifera ligustica* queens produce more brood/bees and their offspring are less winter-hardy (12). However, studies show that environmental diversity may contribute to behavioral and phenotypical traits of honeybees within the same subspecies. Researchers have found significant decreases in honey production and spring build-up in *Apis mellifera ligustica* after relocating queens that originated in geographical and climatically diverse regions in Italy, showing evidence that genotype-environment interactions may relate to colony fitness (13). In another study, researchers found that, after relocating bees to Canada to breed for four generations, several heritable morphological and colony traits, such as forewing size, contributed to increased honey production (Szabo, 1987).

There has also been recent evidence that honeybee-environmental interactions may relate to colony fitness on the cellular level. In a 2010 study, researchers studied queen bees from Canada, NZ, Hawaii, California, and Chili and compared their mitochondrial activity. Mitochondria activity of the northern (Canadian) bees was consistently greater than that of the southern bees, and the authors suggested that an increase in energy production is correlated with fitness (Parker et al., 2010). Moreover, findings are showing that unsuccessful overwintering of colonies is associated with reduced expression of immune genes and higher susceptibility to viral infections (Martin et al., 2015). Since southern bees do not experience harsh, northern winters and are likely less adapted to overwintering, these bees could be more susceptible to local strains of viruses and other pathogens than northern bees. These studies suggest that environmental adaptations over several generations in *A. mellifera* influence foraging behavior and molecular survivability factors that are specific to a northern climate. Such factors are heritable and a product of local queen and drone genotypes.

My null hypothesis for this study is that the offspring of locally raised queens from the Northeast are equally adapted for survival and productivity in this region as compared to queens and worker bees from California. I chose California-raised animals for the second population because the climate is much different from that of the northeast and because California is home to some of the largest commercial queen breeders in the world.

Factors related to colony fitness include collection of nectar and pollen, brood rearing, resistance to pathogens, and winter survivability. To test my hypothesis, we examined these factors (choosing five of the most common and relevant pathogens in honeybees). The measures that we specifically chose for the productivity/foragability component of this project were colony weight/growth, weight of pollen collected from each hive, and the population of brood in each hive. The pathogens that we measured were *Varroa destructor* (Varroa mites), *Nosema sp*., and three common RNA viruses – deformed wing virus (DWV), black queen cell virus (BQCV), and Israeli acute paralysis virus (IAPV).

Change in colony weight is a common measure of productivity of hives, as it accounts for the total biomass of that colony, which includes honey/nectar, pollen, comb, and bees and brood (15). A more prolific hive will have a greater growth rate when compared to a less prolific hive. Collecting pollen is another method to evaluate colony productivity (15). Pollen stores in a honeybee colony are used to make bee bread (a dietary component for older brood) and aid as a protein source for adult bees (18). Keeping track of this resource gives an idea as to how efficient a colony is at rearing brood and preparing for winter. Quantifying the population of brood (larvae) in a colony is another effective measurement of fitness/productivity as it allows one to evaluate both the egg laying efficiency of the queen as well as that of the nurse bees (16). Notwithstanding the fact that a more active reproducing female helps develop a larger colony, the population of eggs and brood is a limiting factor in controlling the percent of foragers that collect pollen (17). The quality of the queen, therefore, is partially determinant for the overall productivity of the hive.

The five pathogens that we examined are some of the most prevalent and detrimental to *Apis mellifera*. The varroa mite alone, since its arrival into the United States in the 1980s has caused devastating colonies losses throughout commercial hobbyist apiaries. This parasite impacts honeybee health on both the individual and colony level (22,23,24). The varroa mite’s life cycle begins in the cell of a honeybee larva where it feed on the hemolymph of its host (20). When the adult bee emerges from its cell, and varroa that are present emerge with it and can then come in contact with other bees. This makes *V. destructor* an extremely contagious parasite. Additionally, recent studies have shown evidence that this ectoparasite also acts as a vector for deformed wing virus and other RNA viruses (19, 21). A colony that is more resistant to varroa infestation on account of hygienic behavior, therefore, would also be less susceptible to certain RNA viruses.

The RNA viruses we looked at (DWV, BQCV, and IAPV) are the three most common viruses in honeybees (25). These viruses can have morphological symptoms on the host (crippled wings and darkened larvae for DWV and BQCV), but IAPV also have been shown to lower mitochondrial activity (28, 26, 25). Honeybee viruses have been linked to lethal damages on the colony level, and a colony that is more immune to these viral infections would be more productive and have much better chance at winter survival (27).

The final pathogen we quantified was the microsporidian *Nosema*. The two species of *Nosema* found in *A. mellifera* are *N. ceranae* and *N. apis*. While both species are similar in that they both infect the ventriclulous of the host animal*, N. ceranae*, the most common of the two spores, is also the more lethal of the two species (34, 35) . Studies have shown that adult workers can die after 8 days of exposure and that nosemosis and causes reductions in colony size as well as reduced honey production. (33, 32, 31). Other studies have shown that colonies *N. ceranae* has also been shown to induce immunosuppression of particular immune genes in infected animals, which could also increase susceptibility to other pathogen infections. (29, 30). Strains of *A. mellifera* that are less susceptible to this infection and have heightened expression of immune genes should be more fit for survival where the disease is prevalent. In general, this concept of localized immunity was largely applied to our study, which referred worker genotypes back to the geographical origin of queen bees.

**MATERIALS AND METHODS**

**Selecting & Requeening Colonies**

Forty *Apis mellifera* colonies were selected at random from an apiary of over one hundred 8-frame single story Langstroth hives. The owner of the colonies, a commercial migratory beekeeper with yards in South Carolina and central New York, added an additional 8 colonies to the sample in order to test his own queens for viruses and *Nosema* loads. The forty colonies were dequeened on 5/30/16. At that same time, we equalized the hives by exchanging frames of brood where necessary. The majority of the colonies had 4 full frames of brood while only a four hives were given three. The population size of each colony was also relatively equalized at the start of the study. Three days later, the hives were then moved to their wintering locations in two of the owners mating yards – 20 hives in each yard. The hives were left on pallets for the remainder of the study. After the colonies had been queen less for a total of 4 days, we requeened 10 colonies in each yard with New World Carniolan queens bought from one of the largest queen breeders in California and 10 colonies in each yard with our “local” Carniolan-type queens shipped in from a reputable queen breeder in Vermont. We numbered the hives using a using a letter/number format, with Y + number indicating the yard number and C/L + number indicating the whether the hive was Californian (C) or Local (L). Because the colonies had been queen less for four days (a shipping/weather delay), when we requeened the hives, we shook off all of the bees on every frame and cut out any started queen cells. On 6/8/16 (five days after requeening), we checked on all of the colonies and found that all but two of the hives had a laying queen – one in each yard. We put new Californian queens in hive# Y1C1 and Y2C5. Y1C1 accepted the new queen. Y2C5 did not accept the second queen, so it was dropped from the study.

**Weighing Colonies & Determining Relative Growth**

We made 4 weight measurements of the colonies – one before the new queens’ offspring had hatched in order to establish a starting weight (6/14/16) and 3 other measurements to establish change in weight/growth over time after the treatment. We weighed the colonies by placing each hive body and super on top of a digital scale mounted on a plywood board. The scale was accurate to 0.2 pounds. These measurements took the total colony weight into consideration, including the weight of the boxes and frames. While this was acceptable to find the relative change in weight over time, we also subtracted the average weight of an empty box to find a more accurate representation of colony weight (bees, brood, honey/nectar, pollen, and comb).

**Measuring Pollen Collection**

Using pollen traps is a standard method for collecting pollen and evaluating productivity of forager bees (16). Ten-frame Betterbee® plastic pollen traps were adapted for 8-frame Langstroth hives by screwing a small pine board to the side of the device. The traps were advertised as collecting less then 50% of the pollen from foragers entering the hive. While the exact percent of pollen removed by the traps was unknown, were will only looking for a consistent amount of pollen collected by the hives so that we could evaluate relative forager productivity between the Local and Californian groups.

For each measurement, the traps were left closed for 24 hours once a week, starting on (6/14/16). After the 24-hour period where that traps were collecting pollen, we closed the traps and collected the pollen from each hive. In order to consistently remove the moisture from the samples, we then put each sample of pollen collected into tared glass dishes and heated them in a drying oven for 24 hours at 45-50 oC. Each pollen sample was finally individually weighed. We also recorded high/low temperatures and cloud cover on collection days for later analysis.

**Counting Brood Combs**

The amount of brood present in each colony was estimated by periodically counting frames of brood. This standard method is somewhat subjective, so we counted frames that were ≥ ¾ full as “full frames.” Those that contained less were added together with other < ¾ frames to equal a frame, a similarar method used in another study (37). The starting number of brood combs was 3-4 in each colony on 5/30/16. We counted brood on five other occasions.

**Quantifying *Nosema* Spores**

Three ¼ cup samples of foragers were taken from each hive at three different intervals throughout the study: before the experimental queens’ offspring started hatching (6/16/16) and two times after new bees had hatched for several weeks (7/19/16 and 8/9/16). Since *Nosema* spore infections are most present in foragers, we collected bees from peripheral combs containing fresh nectar and pollen.

We then counted out fifty whole bees from each sample and homogenized the abdomens in 50 mL of distilled water using a tube and pestle for 1 min (36). We loaded each sample into both wells of a hemocytometer and counted all the *Nosema* spores in the center and corner squares of the middle grid under phase-contrast light microscopy (x400) – a standard method (36). We were finally able to calculate the number of spores per bee/mL for each sample (36). It should also be noted that we were not able to tell the difference between *Nosema apis* and *Nosema ceranae* using this method.

**Determining levels of *Varroa destructor***

Varroa mite levels were evaluated four times throughout the duration of the experiment using the powder sugar method on a mite per 100 bee metric (16). One quarter cup of bees (about 300 bees) was sampled from the brood chamber of each hive and put in a screened mason jar. We then let the bees sit for 60 seconds in the jar after dropping 1 tablespoon of powdered sugar on top of the animals. We shook the jar over a white plate that was protected from the wind in shielded chamber until the sugar and/or mites stopped falling – about 30 seconds. Dissolving the sugar with a water spray bottle allowed us to easily count the mites on the white background, giving us a mite/300 bee ratio for each colony. Equipment was disinfected in-between each colony sampling. The owner of the colonies treated for *Varroa* after the first mite count on 6/15/16 using 75 mL of 1.11 specific gravity formic acid, bringing the average down to about 1 mite/300 bees for each yard. This gave us an even starting point for the remaining mite counts.

**Detecting RNA Viruses using PCR**

We determined the presence of deformed wing virus (DWV), black queen cell virus (BQCV), and Israeli acute paralysis virus (IAPV) in each of the colonies using conventional PCR. Fifty-bee samples were taken from the brood chamber of each hive twice throughout the study – one sample before the experimental queens’ offspring had hatched (6/15/16) and one sample after the new bees had been hatching for 15-19 days (7/13/16).

Since the viruses evaluated were RNA viruses, all samples were stored on

-80 oC until RNA extraction/purification. We extracted/purified the RNA using a modified version of the National Honey Bee Survey’s (NHBS) Protocol-04 (Qiagen) (). We used cDNA synthesis reagents from GoScript with random primers. After cDNA synthesis, we ran each sample through a PCR agarose electrophoresis gel using USDA primers for DWV, BQCV, IAPV and *A. mellifera* actin (Table 1). Because the company’s suggested annealing temperatures were significantly different for each primer, we ran each virus in a different gel under different PCR conditions (). We ordered G-Blocks® of the primer amplicons for positive controls for each virus using the USDA primers to find the amplicon of interest within each viral genome.

Table 1. Primers used for the amplification of RNA viral amplicons and *Apis mellifera* actin gene.

|  |  |  |  |
| --- | --- | --- | --- |
| Primer | 5’ to 3’ Sequence | Product size (bp) | Reference? |
| DWV-F | TTCATTAAAGCCACCTGGAACATC | 136 | USDA? |
| DWV-R | TTTCCTCATTAACTGTGTCGTTGA |  |  |
| BQCV-F | TTTAGAGCGAATTCGGAAACA | 140 | USDA/(VanEngelsdorp, Evans et al. 2009) |
| BQCV-R | GGCGTACCGATAAAGATGGA |  |  |
| IAPV-F | CCATGCCTGGCGATTCAC | 203 | USDA? |
| IAPV-R | CTGAATAATACTGTGCGTATC |  |  |
| AmeActin-F | TGCCAACACTGTCCTTTCTG | 155 | USDA |
| AmeActin-R | AGAATTGACCCACCAATCCA |  |  |

**RESULTS**

**DISCUSSION**

**Background & Origin of Study**

I have been keeping bees in Vermont since 2004, starting at the age of 10. My grandfather introduced me to beekeeping, and by the time I was old enough to have my own hives, I started out by purchasing Carniolan queens and bees from a beekeeping supply company that purchased their animals from a breeder in California. As queen bee prices started to rise, I eventually began grafting my own queens and raising bees from local stock. I immediately noticed that the bees I had raised seemed to fair better than the queens I had always bought from California. My bees seemed to come through the winter better, and the colonies seemed to grow at a faster rate in the spring. The offspring of the local queens I raised also seemed to be more prolific foragers, in a side-by-side comparison with some of my CA hives.

Although these observations in my apiary were by no means based off of a controlled experiment, I began to wonder if the geographical origin of queens and the drones they mated with had a direct effect on the behavior of the worker bees on account of environmental adaptations. In other words, I questioned if the offspring of my California queens were less adapted for survival in the northeast than the bees that had be living in the northeast for many generations. Perhaps the warm, dry Californian climate produced bees that were less acclimated to foraging in colder/wetter climates. Maybe the CA bees were “unaccustomed” to collecting from the different flower sources found in the northeast. I finally considered the possibility that local bees were more resistant to certain local strains of pathogens, such as *Nosema* and bee viruses.

I then began questioning local beekeepers in my area about my idea of local bees being more fit than imported Californian bees. Most of them agreed with my hypothesis and had also noticed local bees surviving the winter better and/or being more productive than CA bees. I additionally found some clues in the literature that also suggested that the geographical origins of bees also have an effect on the genotype and behavior of these animals, which can also be translated into factors related to fitness. At this point, I began to design a controlled experiment that could compare these two “strains” of bees side by side.

Upon transferring to Hamilton College to finish my undergraduate work, I approached the Biology department and Professor Herman Lehman became interested in the study I had designed was instrumental in making the project come to fruition by advising me, helping me find funding, and setting the project up in his research lab. Because this project would look at a lot of different variables related to honey bee fitness, Lehman advised that I find a partner to help with field/lab work. Fiona McLaughlin eventually joined the project. We officially started work in May of 2016

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