



Proceedings of the 2018 American Bee Research Conference

Michael Simone-Finstrom

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Proceedings of the 2018 American Bee Research Conference

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Introduction

The 2018 American Bee Research Conference (ABRC) was held January 11–12, 2018 at the Grand Sierra Resort, Reno, NV. Over the two-day conference, a total of 55 oral presentations and 12 poster presentations were given, representing work done from over 30 different research groups from throughout the United States. ABRC was held concurrently with the 2018 American Beekeeping Federation Conference & Tradeshow.

The following are the submitted abstracts for presentations given at the 2018 American Bee Research Conference.

Keywords: *Apis mellifera*, honey bee biology, apiculture

Abstracts of Oral Presentations

1. The egg as an indicator of queen quality and colony stress

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The queen is the sole reproductive female in a healthy honey bee colony. The presence of a healthy and high-quality queen is a key factor in colony survival. Queen quality is a quantitative and qualitative measure of a queen's reproductive potential, which can be influenced by her genetic background, her mating success, as well as her developmental and current environment. Non-destructive assessment techniques for measuring the quality of queens have been proposed but more research is needed. The quantitative and qualitative characteristics of eggs may indicate queen quality and/or colony stress status. Some early work indicates significant variability in the size of queen-laid eggs (see Figure 1), warranting further studies in the context of the current honey bee health crisis. In this work, we investigated the relationship between queens and their egg size. In the first study, we compared the egg size of 22 queens from 6 different stocks of honey bees in the US to assess inter-individual and stock variation. Preliminary results show that stocks vary significantly in egg size despite significant variation among queens within stocks. We also explored the effect of colony population size and colony nutritional status on the size of queen laid eggs. Colony size was negatively related to egg size and queens in food stressed colonies produced larger eggs than control queens. These counterintuitive results indicate that stressful conditions may lead to increased egg size. In conclusion, egg size differs systematically between individuals, among stocks and different colony conditions, which suggest that egg size may be actively controlled by the queen

despite potential genetic differences. The function of egg size variation is not clear yet. Therefore, more studies need to be performed to understand this understudied aspect of honey bee biology.

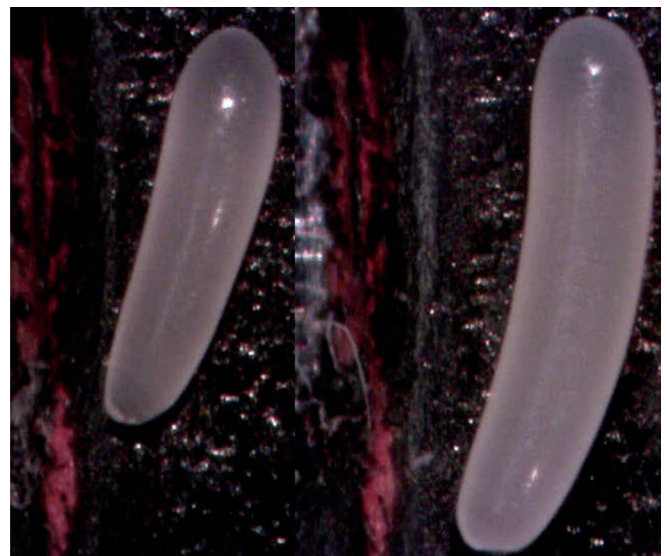


 Figure 1. Egg size variation in the honey bee, *Apis mellifera*.

2. Probing deeper into flowers: Phytochemicals and honey bee health

H.S. Arathi¹, Elisa Bernklau², Alison Hogeboom¹, Louis Bjostad².

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Honey bee populations are experiencing serious challenges ranging from pesticide exposure, habitat degradation, pests and pathogens. Even as the demand for commercial honey bee pollination services continues to grow, the ecological impacts of modern agriculture on bee health with its intensive cropping practices, herbicide tolerant crops, extensive single variety monocultures etc., are increasing causes

for concern. Widespread and indiscriminate application of herbicides has eliminated remnants of native prairies and wildflower patches in marginal and underutilized areas of the farm. The consequent drop in the diversity in pollen/nectar diet of bees, compromises their ability to cope with environmental challenges. In addition, drought stress in plants affects nutrition quality.

Insect chemical ecology may hold answers to some of the problems honey bees encounter in the current agro-ecological realm. The longstanding plant-pollinator mutualism not only reiterates the role of plant chemicals in sustaining this relationship, but also implies that these phytochemicals are of significant importance to bees, as they depend solely on plant products for survival. Although there are advances in knowledge of chemical signals mediating in-hive and bee-pathogen interactions, sufficient progress has not been made in exploring the roles of phytochemicals in regulating honey bee health. Specifically, nectar and pollen contain phytohormones, pyrodoxines and phenolics that stimulate innate immune responses, up-regulating detoxification genes in honey bees.

Leading off from our preliminary analyses that showed significantly lower amounts of p-coumaric acid, a phenolic compound, in pollen collected from colonies foraging on agricultural crops such as canola, we undertook a detailed study to determine whether structurally similar phytochemicals elicit similar beneficial responses in honey bees. We found that 8-day old worker bees maintained on a diet of 20% sucrose solution supplemented with four phytochemicals: caffeine, p-coumaric acid, gallic acid and kaempferol, showed increased longevity and enhanced survival probabilities (Figure 2). However, the magnitude of those effects depended upon the dosage of the chemical. For example, kaempferol was beneficial in high doses and caffeine was only beneficial at low doses. Our results indicate that diversity in nectar and pollen sources could provide honey bees with important nutrients that

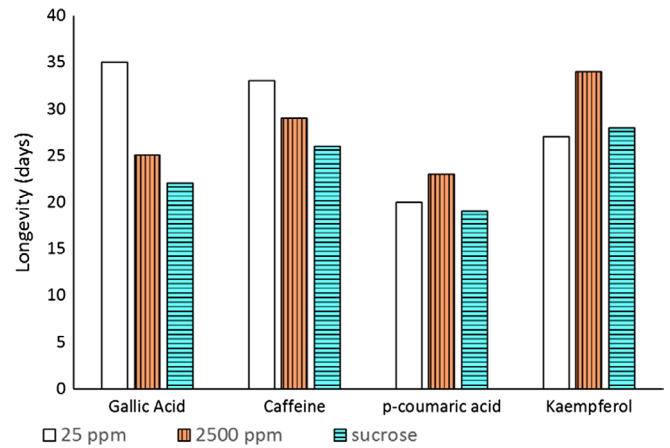


Figure 2. Longevity (days) of bees maintained on dietary supplementation of beneficial phytochemicals.

improve longevity. When access to diverse nectar and pollen sources are limiting, results of our study will provide appropriate management guidelines for dietary supplementation to ensure healthy honey bee hives. Ongoing research is exploring the benefits of these phytochemicals for pathogen and pest tolerance. Reinstating the benefits of plant-pollinator interactions could help sustain the mutualism that is currently being threatened by anthropogenic factors.

3. Comparative characterization of virus content and resistance in different breeds of US Honey Bees

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Recently, honey bees have experienced major population declines throughout the world, including 30% annual losses within the US. Multiple factors play a role in driving these colony losses, ranging from the spread of pathogens and increasing pesticides applications to habitat loss and climate change. Viruses play a major role in causing a high mortality rate in honey bees. *Israeli acute paralysis virus* (IAPV), one of the 22 known bee viruses, can be transmitted by the parasitic mite *Varroa destructor* and is responsible for collapse of some honey bee colonies. Through this study, natural variation to IAPV resistance in honey bee workers of different genetic stocks was assessed. Honey bee queens representing different US genetic lines of bees were obtained from multiple sources in the US as follows: USDA-Pol, USDA-Russian, Italian Californian, Minnesota Hygienic and Italian and Carnolian Hawaiian bees. Upon arrival, four queens from each source were directly sacrificed and frozen in -80°C freezer to be tested for viral load. The remaining queens from each source were installed in small mating nucs. Upon completion of the 21-day brood cycle, combs containing worker offspring from different colonies were transferred before emergence into an emergence incubator (33°C , 60% rel. hum.).

After emergence, workers from each source (mixed ages usually 3–5 days old) were collected in a cup equipped with food (sugar candy) and water. On the third day, all workers were inoculated with IAPV by topical applications of purified virus solutions [diluted 10^{-2}] after shaving of the thorax hairs in triplicate. Two groups, treatment (IAPV-treated) and a control (water) were maintained separately in an incubation chamber (30°C , 45% rel.hum.) for survival analysis. Worker survival was compared among treatment and control groups to indicate relative IAPV resistance of the bee stock and distinguish between the most resistant and least resistant colonies. A total of 5,500 worker bees have been analyzed for this part of the study. This study helped characterize the most and the least resistant stocks to IAPV, thereby clarifying whether virus resistance contributes to the overall health of these strains and provide insights in how the breeding of these lines can be improved in the future for beekeepers and bee-breeders all around the US for raising virus-resistant honey bee stocks. Furthermore, this work is paving a way for selecting important markers for virus resistance that could be employed in breeding efforts to minimize the losses in honey bee colonies owing to IAPV.

4. Honey bee food glands can be affected by neonicotinoids

Selina Bruckner^{1,2,3}, Lars Straub^{2,4}, Laura Villamar-Bouza^{2,3}, Peter Neumann^{2,4}, Geoffrey R. Williams^{1,2,4}.

¹Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, USA. ²Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland. ³European Food Safety Authority, Parma, Italy. ⁴Swiss Bee Research Center, Agroscope, Bern, Switzerland.

Hypopharyngeal glands (HPGs) of mature worker honey bees (*Apis mellifera*) are responsible for the production of royal jelly that is used to feed developing individuals. Neonicotinoids, systemic insecticides widely used for insect management, are suggested to negatively affect HPGs. However, it is unknown how timing of exposure affects HPG

development. Based on previous literature, we hypothesized that field realistic concentrations of neonicotinoids would negatively affect HPGs, and that negative effects on individuals both developing (i.e., eggs, larvae, pupae) and residing (i.e., adults) during pesticide exposure would be strongest.

To test our hypotheses, we employed a cross-foster experimental design. Fourteen queenright colonies were randomly allocated to either control or neonicotinoid treatment groups. For 49 days, a pollen paste was fed to the colonies used to rear experimental workers; the pollen paste fed to the neonicotinoid group was spiked with 4.9 ppb thiamethoxam and 2.1 ppb clothianidin. At adult emergence, half of the experimental workers from each colony were transferred to another colony within the same treatment (Control to Control or Neonicotinoid to Neonicotinoid), and half to another colony of the opposite treatment (Control to Neonicotinoid or Neonicotinoid to Control). Experimental workers were recaptured 8 days post-emergence, the typical age of nursing, for HPG examination (Figure 3).

We found that size of HPGs was negatively affected by neonicotinoids. Moreover, individuals both developing and residing (Neonicotinoid/Neonicotinoid) under insecticide exposure exhibited the smallest HPGs. These data suggest that impaired HPG development due to exposure to field-realistic concentrations of neonicotinoids may be an important risk factor for honey bee health. Considering the importance of HPGs to colony development and queen health, pesticide risk assessment schemes should consider HPG quality in the future.

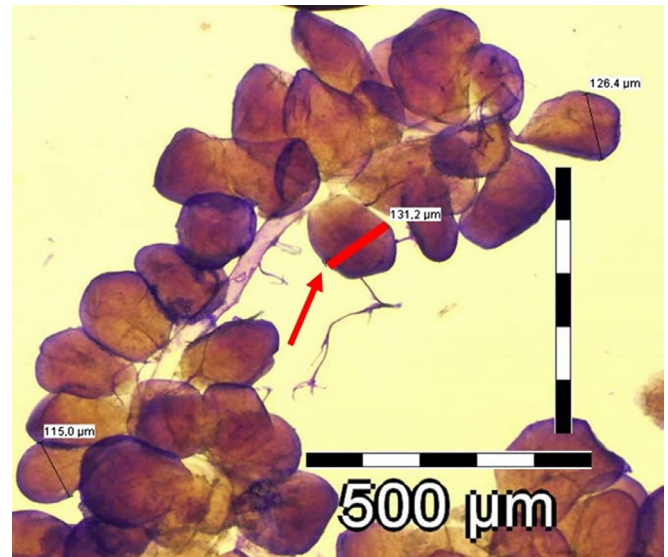


Figure 3. Example of measurement of an acinus to determine the effect of neonicotinoid exposure on hypopharyngeal gland development.

5. Defining the path to the genius hive

Jospeh Cazier, James Wilkes, Edgar Hassler.

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There are many things that technology can do to help advance the health and vitality of the honey bee. This presentation discussed the potential of modern technology in fields like analytics, software development, machine learning and artificial intelligence to lead to better

beekeeping. Eventually this technology can lead to the development of a genius hive, one that can tell you what it needs to be more successful. We outlined the path to building a genius hive, progress that has been made in this direction, and where to go next.

6. The importance of phytosterols in honey bee nutritional physiology

Priyadarshini Chakrabarti, Ramesh Sagili.

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With the United States being one of the largest centers of commercial beekeepers, honey bees ensure sustenance of the billion dollar industries of beekeeping and commercial crop production. Recent alarming honey bee colony losses due to a multitude of factors, such as pests and pathogens, poor nutrition and pesticides, have affected both beekeepers and growers, especially as both are interdependent for their economic sustainability. Pollen forage and protein supplements provided by the beekeepers form the backbone of bee nutrition. In light of colony losses and reported adverse effects on bee health and pollination services, it is crucial to better understand the optimal nutritional needs of honey bees. Unlike other organisms, insects are unable to synthesize sterols. Sterols, particularly 24-methylenecholesterol, are key to honey bee growth and survival, as a number of bee life processes are dependent on the availability of optimum sterol concentrations in their diets. A gap in knowledge regarding the needs of phytosterols in honey bee nutrition has called for urgency in studying the critical roles that sterols

play in the honey bee life cycle. An artificial diet was formulated and supplemented with different concentrations of 24-methylenecholesterol in order to evaluate preferences for sterol content by honey bees in laboratory cage experiments. Our study suggests that honey bees preferentially consume diets rich in sterols compared to control diets and diets containing low concentrations of this particular sterol. Bees fed sterol diets survived significantly longer than those fed control diets without sterol. Additionally, the total head protein content in bees from the high sterol treatment groups was significantly higher than in bees exposed to low or no sterol diets (control). Labeling the sterol diet with isotope also helped us quantify the isotope and trace the diet through the adult honey bee tissues. The results obtained from studying honey bee abdominal fat contents and specific honey bee head proteins (proteomics study using mass spectrometry) also corroborate our other findings.

7. Fat body lipolysis connects poor nutrition to hypopharyngeal gland degradation in *Apis mellifera*

Vanessa Corby-Harris, Lucy Snyder, Charlotte Meador.

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The hypopharyngeal glands (HG) of honey bee nurse workers secrete the major protein fraction of jelly that is fed to developing larvae, other worker bees, and queens. Poorly nourished nurses have small HGs, which actively degrade due to ecdysteroid hormone induced

programmed cell death. To better connect nutritional stress with HG degradation, we looked to other insect systems, where different types of stress often result in fat body degradation (i.e., lipolysis). The fat body contains stored cholesterol and cholesterol is a substrate for ecdysteroid

synthesis, and so we tested whether starvation caused fat body degradation and the liberation of cholesterol into the hemolymph. Ecdysteroid signaling and response pathways have been implicated not only in HG degradation, but also in fat body mobilization. So we tested whether and where genes in this pathway were upregulated. We find that, as expected, nurse bees deprived of pollen have smaller HGs with decreased function and increased signatures of tissue degradation and cell death. Starved nurses also exhibited increased fat body lipolysis, increased hemolymph cholesterol levels, and increased expression of ecdysteroid production and response genes in the head. We did not observe increased expression of these genes in the fat body. These data support the hypothesis that nutritional stress induces fat body lipolysis, which liberates cholesterol. This cholesterol most likely fuels ecdysteroid production in the head, which then leads to HG degradation. Our results show how poor nutrition leads to reduced nurse health and function (see Figure 4). Our work also suggests links between HG degradation that is induced by starvation and other stressors, such as temperature extremes and infection, which may act synergistically to compromise nurse health and function.

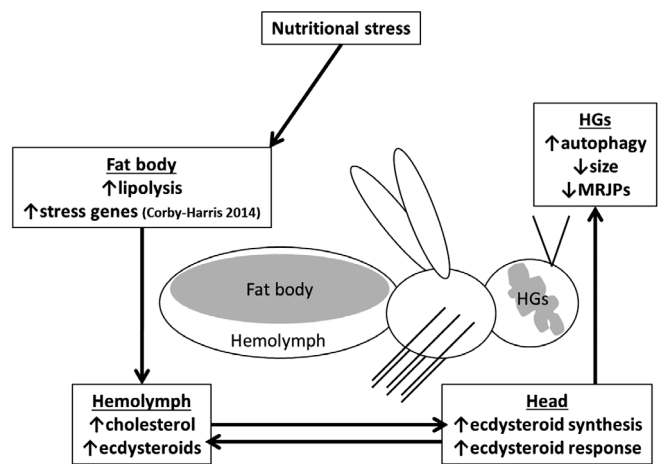


Figure 4. Overview of effects of nutritional stress on health of nurse honey bees.

8. The *Tropilaelaps* mites threat: Observations of their reproductive success

Lilia I. de Guzman¹, Patcharin Phokasem², Kitiphong Khongphinitunjong³, Amanda M. Frake¹, Panuwan Chantawannaku^{2,4}.

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Tropilaelaps spp. are more successful parasitic mites of *Apis mellifera* than *Varroa destructor* in Asia (Burgett et al., *Bee World* 64: 25–28). We sought explanations to this increased success by assessing *tropilaelaps* mite fecundity on European bees in three short experiments using the mite transfer technique: (1) fecundity in brood cells with normal wax cappings and recapped brood; (2) fecundity of foundresses collected from newly sealed larvae (NSL) and tan-bodied (TB) pupae and inoculated into NSL within 5 hours of mite collection and also in naturally infested brood; and (3) fecundity of foundress and daughter mites collected from TB and inoculated immediately into 4th instar larvae (L4) sealed with gel caps (de Guzman et al., 2013 *J. Apic. Res.* 52: 262–263). The fecundity of foundress *tropilaelaps* was higher in brood cells with normal wax cappings (3.27 ± 0.27 progeny) than in recapped brood (1.59 ± 0.11 progeny). Likewise, foundress or daughters that were deliberately inoculated into brood cells also produced about two offspring each, which corroborated other findings (reviewed in de Guzman et al., 2017 *J. Econ. Entomol.* 1–14). It is interesting to note that about 75% of the inoculum daughter mites with or without males in their natal cells reproduced, with some producing both males and females. This mite transfer technique, which imitates the release of mites when infested brood is opened by bees and the eventual re-invasion of exposed mites into suitable hosts, suggests that hygienic activities may disrupt or delay *tropilaelaps* mites' reproduction. This was indicated by the higher proportion of foundress mites that did not reproduce in the recapped brood than in undisturbed brood cells, and the increased presence of nymphal stages in the TB-NSL group (see Figure 5). Nonetheless, hygienic activities and increased production of

daughters only (see Figure 5(b)) may have less of a negative impact on *tropilaelaps* mite population growth since both foundress and daughter *tropilaelaps* successfully reproduced immediately without spending a phoretic period on adult bees. These observations may help explain the competitive advantage of *tropilaelaps* mites over *varroa* mites in Asia.

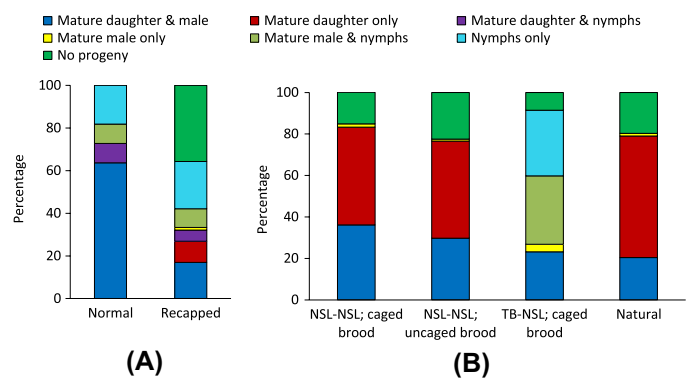


Figure 5. Reproduction parameters of foundress *tropilaelaps* mites: (a) in brood cells with normal wax cappings and recapped brood; and (b) in brood cells inoculated (within 5 h) with foundress collected from newly sealed larvae (NSL) and tan-bodied (TB) pupae and inoculated into NSL.

9. Drone brood removal: A bee-utiful form of *Varroa* control and source of edible insect protein

Bridget Gross¹, Reed Johnson²

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In 2013, the Food and Agriculture Organization of the United Nations published a report urging Western Nations to begin consuming insects. Edible insects provide a sustainable, reliable source of protein, and have been widely utilized by developing countries. At the same time, *Varroa destructor* has been plaguing beekeepers. Multiple mite control strategies have been developed, one being drone brood removal. The first goal of this project is to examine the effectiveness of drone brood removal. Five colonies were selected for this experiment, with three receiving drone frames and two receiving regular brood frames. Two frames were inserted, each frame being inserted about one week apart. Alcohol washes were taken prior to treatment, during treatment and after treatment to evaluate how mite levels changed over time. Mite levels did not differ between groups that received drone frames and groups that did not receive drone frames prior to treatment or during treatment, but were different post-treatment in the month of August. From this, it is concluded that drone brood removal is effective. The second goal of this project is to examine the utilization of drone larvae (see Figure 6) as a source of sustainable protein for humans. Beekeepers were interviewed regarding their feelings towards edible insects, views on drone brood removal, and willingness to sell drones. Bug farms, which are places that raise and process insects for human consumption, were interviewed regarding the edible insect market and their willingness to produce drone larvae products. A majority of beekeepers were willing to sell drone larvae, and all bug farms were willing to sell drone larvae. Ideally, a system would be created where



Figure 6. A drone larva removed from the comb. Photo by B. Gross.

beekeepers can sell their larvae to bug farms, and bug farms can process the larvae for consumers. Selling drone larvae essentially incentivizes the use of drone brood removal for beekeepers, as there is potential for beekeepers to earn income by selling drone larvae to bug farms while also controlling for *Varroa* mites.

10. From Bloom to Boom: An investigation of *Monarda fistulosa* var *menthifolia* (oregano de la sierra) for potential bee and human health

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Monarda fistulosa var. *menthifolia*, is a widespread North American native plant (also known as bee-balm, bergamot, or oregano de la sierra) that possesses a similar chemical profile to oregano including carvacrol, thymol, α -pinene, β -pinene, sabinene hydrate, α -terpinene, citronellyl acetate, and β -caryophyllene (Zamurenko, et al., 1989) Specific to bee health, thymol has been used to successfully control *Varroa* mites and prevent fermentation and the growth of mold in bee colonies (Calderone, 1999) and is already commercially available (Floris, 2004). In addition, essential oils of oregano have been tested as a supplement to realize the same effects (Sammataro, et al., 2009).

Our research evaluated *Monarda* as a habitat-enhancing plant by assessing the presence and relative concentration of thymol and carvacrol in nectar, honey, and hive architecture while *Monarda* is flowering; and afterwards to determine the persistence of the chemical constituents and evaluate its effects on bee health. Objectives include analysis of *Monarda* nectar, honey and pollen using gas chromatography to determine volatile compound content; and to determine *Nosema* and *Varroa* mite counts in hives with access to different diets.

Colonies were established from 3 lb packages with sister queens (ZQB-CA) and were initially fed unbleached, non-gmo pure cane sugar (Zulka) 1:1 syrup at NMSU-ASC in mid-May before being placed in an oregano field (see Figure 7). Paired honey bee colonies were placed at the three distances (~0 m, ~160 m and ~2,400 m) near the *Monarda* field in Embudo, NM. Another pair of colonies was placed under a net (4 m x 8 m) for concentrated *Monarda* foraging. In addition, a pair of colonies was placed in NMSU-ASC with no exposure to *Monarda*.



Figure 7. R. Heyduck (NMSU-ASC) and M. Kirby (ZQB) collecting sample from research hive in *M. fistulosa* (Oregano de la Sierra) bloom. Photo by Jane Moorman.

Post oregano bloom, all colonies were moved to high elevation apiary in Truchas, NM on 24 June 2017. *Nosema ceranae* spore loads, *Varroa*

mite counts, as well as presence of deformed wing virus, Chalkbrood disease and European foulbrood were conducted by BIP (Bee Informed Partnership) program at three points before/after exposure to *Monarda*. Samples were taken 4 June, 22 June, and 24 July. *Monarda* bloom occurred late May-mid June.

Chemical analysis of honey gathered by bees, conducted by cooperators at San Juan College, Farmington, NM, will hopefully shed light on the volatile compounds present in the honey and their concentration. Continued tracking of colony health will determine the impact, if any, of exposure to oregano.

11. Effects of insecticide-fungicide combinations commonly applied to almonds during bloom on honey bee larval development

Chia-Hua Lin, Andrea Wade, Colin Kurkul, Reed Johnson*.

Department of Entomology, The Ohio State University, Wooster, OH, USA. *Presenter.

The almonds in California's Central Valley rely on approximately 2 million honey bee colonies placed there during bloom to provide pollination services. Pollinating beekeepers have reported observing dead adult bees and dead or malformed brood during and after almond bloom, which are attributed to pesticide exposure. The insecticides used during almond bloom are not known to cause harm when bees are exposed to the active ingredients at field-relevant levels. However, these insecticides are frequently applied as a tank mix with a fungicide and the consequences of exposure to such combinations are largely untested. The objectives of this study were to test the effects of the most common insecticides and fungicides applied during almond bloom on honey bee larval development in a laboratory bioassay. *In vitro* rearing of worker honey bee larvae was performed to test the effect of insecticides (chlorantraniliprole, diflubenzuron, and methoxyfenozide) and fungicides (propiconazole, iprodione, and boscalid-pyraclostrobin mix), applied alone or in insecticide-fungicide combinations, on larval development. Newly hatched (~24 hr old) larvae were fed with diets artificially contaminated with active ingredients from the above combinations at maximum label rate ratios. Overall, larvae receiving insecticide and insecticide-fungicide combination treatments were less likely to survive and develop into adult bees compared to the control and fungicide-only treatments. Chlorantraniliprole significantly increased larval mortality and the negative effect was amplified when the insecticide was combined with propiconazole or iprodione. These combinations were applied to approximately 30,000 acres of almonds during bloom in 2014 (Figure 8). Diets containing diflubenzuron generally increased larval mortality although diflubenzuron-fungicide combinations showed similar effects as the insecticide alone. Approximately 45,000 acres of almonds were treated with diflubenzuron or the related insecticide buprofezin during bloom in 2014. No significant effect on larval mortality was detected with methoxyfenozide or any of the

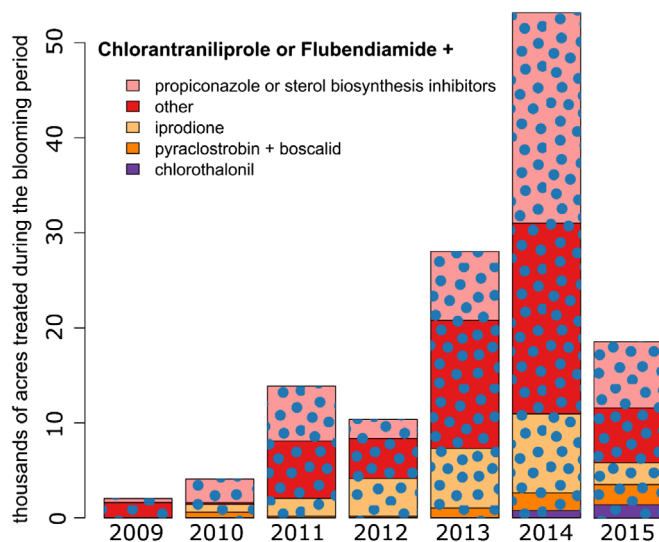


Figure 8. Area of almonds treated during the blooming period with a ryanodine receptor agonist insecticide (Altacor, Belt or Tourismo) tank-mixed with a fungicide. The blooming period is defined as 15 February to 15 March. Data from the California Department of Pesticide Regulation "Pesticide Information Portal" (<http://calpip.cdpr.ca.gov>).

methoxyfenozide-fungicide combination treatments. These results indicate that exposure to insecticides applied during almond bloom has the potential to harm the health of honey bee colonies. The effect of exposure to certain insecticides may be more damaging when the insecticide is applied as a tank mix with fungicides.

12. Sentinel Apiary Program: Three years of pollen pesticide results

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Since 2015, the Sentinel Apiary Program has helped stationary beekeepers better monitor and manage their colony health. With monthly *Varroa* and *Nosema* testing and continuous collection of hive scale data all shared publicly online (www.beeinformed.org/programs/sentinel), Sentinel apiaries act as a benchmark for bee health for entire regions of beekeepers. The program has grown every year, with 2017 consisting of 68 participants from 26 states across the US. This represents almost 500 colonies with consistent sampling and management data. We are now beginning to use these data to link colony health, management, parasite loads and pesticide exposure over time.

In the program's home state of Maryland, participants have the opportunity to submit bimonthly pollen samples for pesticide residue analysis coordinated by the Maryland Department of Agriculture (MDA). From 2015–2017, 183 pollen samples were processed. Using hazard quotients (HQ) as a proxy for colony risk from pesticide exposure (Traynor et al., *Sci. Rep.*, 2016, 6: 33207), various analyses were conducted. Taking into account both the toxicity of a pesticide to honey bees (LD₅₀), as well as the level of exposure to the colony (ppb), the HQ allows us to assess the potential risk posed to a colony by the real, in-field levels of exposure.

Almost half (40%) of samples contained zero pesticide residues. The highest number of products detected in a single sample was eleven. This sample also had the highest HQ of any sample at 45,000 (HQ > 10,000 is considered probably harmful to colony health). Almost all of this sample's high HQ (99.9%) was due to by extreme levels of Thiamethoxam (>890 ppb), a neonicotinoid with very acute toxicity to bees. While only 4% of all samples contained neonicotinoid residues (see Figure 9), their high toxicity can still pose a threat to colony health. We are focusing on these high risk samples to work directly with beekeepers and growers to prevent such exposures in the future. We are also looking to identify peak pesticide risk periods for beekeepers near different land-use types so they can be proactive with their management. Continued pesticide sampling and participation in monitoring programs will provide vital information allowing us to identify such trends.

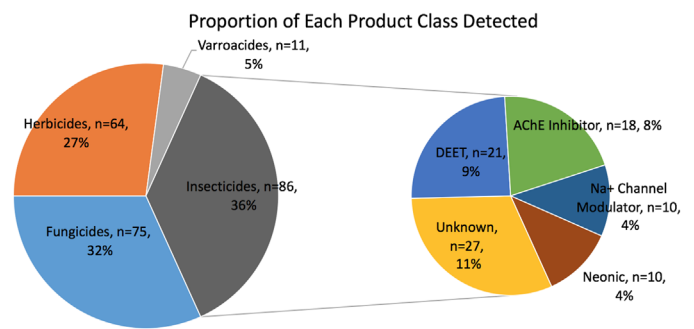


Figure 9. Proportion of each product class detected in three years of samples. The low incidence of varroacides is due to sampling method (pollen captured at colony entrance, not bee bread).

13. A convolutional neural network for recognizing bees in video analysis of forager traffic

Vladimir Kulyukin, Astha Tiwari.

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Electronic beehive monitoring extracts critical information on colony behavior and health without invasive beehive inspections and reduces transportation costs through wireless data transfer. BeePi is a multi-sensor electronic beehive monitor that consists of a raspberry pi computer, a camera, a temperature sensor, a clock, and three microphones embedded in hive walls. All BeePi components are off-the-shelf and fit in a shallow Langstroth super. BeePi was piloted in 2014. In 2015, two BeePi units were tested in Northern Utah in two Langstroth beehives for two weeks. In 2016, four BeePi units were tested in Northern Utah for two months. In 2017, four BeePi units were deployed in Northern Utah for five months (May to September) to collect 150GB of video, audio, and temperature data. A convolutional neural network was developed to recognize bees in 32x32 images. The network's input layer accepts 32x32 images and is connected to a convolutional layer with 32 3x3 feature maps and the rectifier linear unit activation function. This layer feeds to a max pooling layer with a 2x2 pool. The next two layers are fully connected 64-neuron layers with the rectifier activation function. These two layers are followed by a

max pool layer with a 2x2 pool. This layer is connected to a 50-neuron layer that feeds to a dropout layer configured to randomly exclude 50% of its neurons to reduce over-fitting. The output layer has two neurons for the two target classes of bee and no-bee. A sample of 116 30-second videos recorded with a BeePi monitor was taken. Each video was segmented into 745 360x100 frames, and each frame into 3,000 32x32 images. Each image was manually labeled as containing at least one bee (bee) or containing no bee or a small part of a bee (no-bee). 17,000 (8,500 bee and 8,500 no-bee) images were used in training and testing the network (70% for training and 30% for testing). When trained on 50 epochs with a batch size of 25, the network achieves a training loss of 0.4, a training accuracy of 0.87, a validation loss of 0.5, and a validation accuracy of 0.8. When trained on 100 epochs with the same batch size, the network achieves a training loss of 0.01, a training accuracy of 0.99, a validation loss of 0.17, and a validation accuracy of 0.98. The results indicate that convolutional neural networks have potential in video analysis of forager bee traffic.

14. Are bees out for the gains? Honey bee pollen preferences between nutritionally distinctive diets

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Honey bees (*Apis mellifera*) collect pollen as their only natural source of protein, providing a colony with nutrients critical for growth and development. Limitations of few essential amino acids can become a nutritional bottleneck for growing colonies. Studies indicate that pollen intake is correlated to physiological development, such as an individual's hypopharyngeal glands and ovaries. Colonies experiencing a pollen dearth adapt behaviorally by ceasing brood production to avoid rearing malnourished brood. Thus, access to pollen can greatly improve worker population in a colony. Because the nutritional content of different plant species can vary significantly, understanding honey bee pollen preferences is important for improving honey bee nutritional ecology. The goal of this project is to determine a colony's preference between

diets that are nutritionally distinct for protein content to better understand bee floral preferences. Nucleus colonies were established in screened enclosures and starved until pollen stores were depleted in the hive. We performed a choice test between a low protein pollen diet, *Brassicaceae*, and a high protein pollen diet, *Rosaceae*, for each colony. Pollen consumption and observational data were recorded throughout a 5 day period. Interestingly, honey bees strongly preferred the lower protein pollen diet, suggesting that protein content may not be a strong factor for honey bee pollen preferences. Instead, others factors, such as the differences in the distribution of amino acids, color, odor, or pH, may induce a stronger effect on bee pollen foraging preference.

15. Parasite manipulating hosts? *Nosema ceranae* infected honey bee workers are more easily accepted by non-nestmates

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Parasites have been known to manipulate host physiology or behavior so that their own fitness is increased. Richard Dawkins cites many examples of this in his book, *The Extended Phenotype*. One of the examples he cited was that honey bees infected with *Nosema apis* will become foragers earlier and this might be beneficial to the parasite. We have previously shown that even though *Nosema* infection increases juvenile hormone biosynthesis and results in higher hormone titers (Huang & Lin, 2004 Eighth International Conference on the Juvenile Hormones, Scientific Program Abstracts p. 12), this is most likely not host manipulation because infected bees also showed increased higher metabolism.

Honey bee workers show guarding behavior with specialized “guards,” with an average age of ~14 days (Huang et al., 1994 *J Comp Physiol A* 174: 731–739). They “smell” each incoming forager and reject those that do not belong to the same colony. It is possible that *Nosema* might change host “smell” such that infected bees will be more easily accepted by non-nestmates, so that the pathogen may gain entry to a new colony to increase its fitness. The aim of this work is to determine whether honey bee workers infected with *Nosema ceranae* will become more easily accepted by non-nestmates. If so, it will more likely be a case of *Nosema* manipulating the honey bee hosts.

We infected newly emerged worker bees with 50,000 fresh spores by feeding then with 2 microliters of 50% sugar syrup. Control bees were fed with syrup alone. The bees were then isolated in glass vials for 30 min to ensure all food was consumed to reduce *Nosema* spore transmission among bees. The bees were then caged for 7 days in the laboratory. On the 8th day, they were presented to a non-nestmate guard bee using a standard nest-recognition assay (Breed et al., 2004 *Animal Behaviour* 68: 921–928). We repeated the experiment with bees from 3 different colonies, each of them presented to four different recipient colonies.

There was a significant reduction in the percentage of bees rejected by non-nestmates when bees were infected with *Nosema ceranae*, compared to those not infected. We also found a significant colony effect for the colonies that provided the guard bees; the interactions between infection status and guard colony source were also significant. Source of infected bees did not differ significantly in their acceptance. These results suggest that besides other changes made by the microsporidian parasite, *Nosema ceranae* can also reduce aggression toward their host, perhaps to increase their spread to other colonies. The most likely mechanism is a change of hydrocarbons in the infected bees which will be investigated in a follow up study.

16. Methyloomic and transcriptomic analyses of lethal IAPV infection in honey bee pupae

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Pollination is required for approximately 30% of food crops and honey bees (*Apis mellifera*) are the major managed pollinator used in agriculture. Relatively little is known about the mechanistic interactions between honey bees and their pathogens. Israeli acute paralysis virus (IAPV) is an important honey bee pathogen and is thought to be closely associated with colony loss. To further our understanding of its pathogenicity, we conducted Methyl-Seq and RNA-Seq experiments for thorough analyses of pupal methylomic and transcriptomic profiles after IAPV infection. The host transcriptomic responses were characterized across three different time points. These time points include an

early stage of infection in the first round of viral replication (5 hours post-infection), and two later time points (20 and 48 hours post-infection) with the last time point including the initiation of host tissue necrosis. Affected pathways based on the list of differentially expressed genes are antimicrobial peptides, ribosome activity, proteolysis, and protein processing in the endoplasmic reticulum. We identified more than 300 new genes that respond to IAPV viral infection by CpG methylation. This is the first evidence of dynamic epigenetic changes as a potential anti-viral defense mechanism in *A. mellifera*.

17. Honey bees and neonicotinoid-treated corn seed: Pollen contamination, exposure, and effects

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The majority of corn (*Zea mays*) planted in the US is seed-treated with neonicotinoid insecticides, which can become abraded during the mechanical sowing process and dispersed as dust in the landscape (Figure 10). This phenomenon is well documented and has been

associated with bee kill incidents throughout North America and Europe. The objectives of this study were to (1) evaluate the associations between corn planting, honey bee worker mortality and exposure to corn seed treatment insecticides, and (2) assess long-term impacts

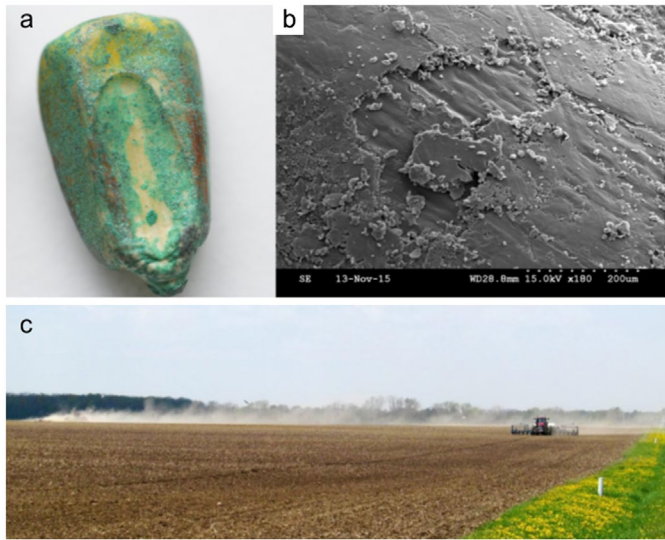


Figure 10. Seed treatments are applied to seeds as flowable solids that dry to form a coating. In corn, this coating results in visibly patchy coverage of the seed (a). The seed treatment forms particles of varying size on the surface of the seed as captured using scanning electron microscopy (SEM) (b). The striated surface visible in the center of the micrograph is the seed surface, uncovered by the broken seed treatment coating. Fragmented particles of the seed treatment coating are then emitted as planter dust during the sowing process (c). Macrophotography was performed by M. Spring, and SEM preparation by K. Kaszas.

of the exposure to corn seed treatment insecticides on honey bee colonies. We examined the association between honey bee worker mortality, exposure to corn seed treatment insecticides, and corn planting at 13 apiaries in west-central Ohio in 2013–2015.

We consistently recorded increased concentrations of corn seed treatment insecticides in bee-collected pollen and elevated worker mortality during corn planting each year. Clothianidin and thiamethoxam, two insecticidal ingredients in corn seed treatments, were the most abundant neonicotinoid compounds detected in bee-collected pollen and the detection of these compounds occurred more frequently (Fisher's Exact Test, $P < 0.0001$) and at higher concentrations during corn planting periods relative to non-planting periods. Average worker mortality was significantly and consistently higher during corn planting than during non-planting periods ($N = 12\text{--}38$ colonies per year; 2-tailed paired t -test, $P < 0.018$ for all comparisons). Additionally, mortality during corn planting was consistently greater than the average mortality throughout the one-month sampling period for all years, suggesting an association between elevated mortality and corn planting. Insecticide concentrations in bee bread sampled immediately after corn planting was strongly correlated with the concentrations in bee-collected pollen sampled during planting (Pearson's $r = 0.79$, $P = 0.03$, $N = 7$ apiaries) but the contaminations had subsided in bee bread sampled two weeks later. We did not observe significant effects of the exposure on brood production, food storage of the colonies in later seasons or winter survival of the colonies.

18. Honey bee host-pathogen interactions at the colony, individual and cellular levels

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Since 2006, honey bee populations in some parts of Europe and North America have experienced high annual losses that are associated with elevated pathogen prevalence and abundance. The majority of honey bee pathogens are positive-sense single-stranded RNA viruses, therefore a better understanding of honey bee-RNA virus interactions at the colony, individual, and cellular levels may result in the development of strategies that mitigate colony losses. Longitudinal monitoring of migratory honey bee colonies that participate in almond pollination demonstrated that time of sampling and beekeeping operation were the best predictors of pathogen composition. Additionally, *Deformed wing virus* (DWV) and *Lotmaria passim* abundance were negatively correlated, while DWV abundance and *Varroa* loads were positively correlated. To further characterize the honey bee antiviral response at the individual level, we performed transcriptome sequencing on bees

infected with a model virus (Sindbis-GFP) in the presence or absence of double stranded RNA (dsRNA), a virus associated molecular pattern (VAMP). Our results indicate that honey bee antiviral defense involves both sequence-specific RNAi and non-specific dsRNA-mediated pathways. This includes genes likely important to virus and dsRNA detection (e.g., helicases), immune pathway signaling (e.g., kinases, transcription factors), and antiviral effectors (e.g., RNAi machinery). Further analysis of the antiviral role of two genes was confirmed in individual bees using gene knock-down. To distinguish general and virus-specific immune responses, we are evaluating *Lake Sinai virus 2* replication and immune gene expression in cultured honey bee cells. Together these studies will further elucidate the mechanisms of honey bee antiviral defense and improve our understanding of viral infections at the colony, individual and cellular levels.

19. Exploring within-day hive weight data

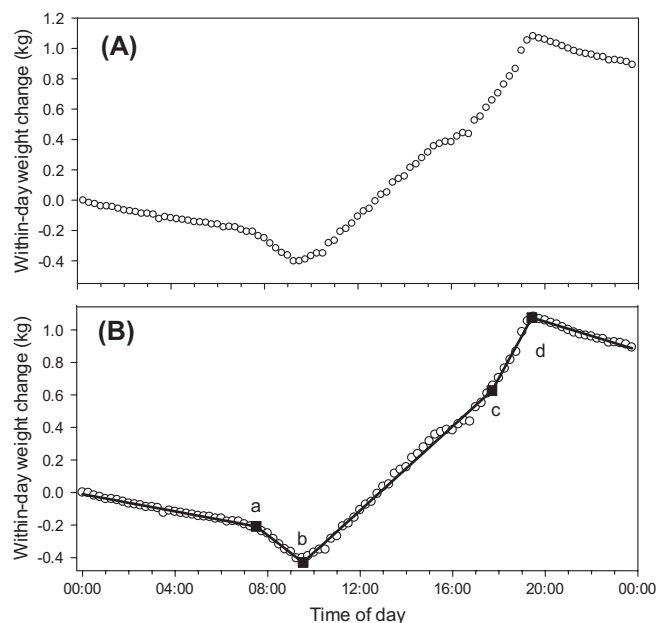
William G. Meikle¹, Milagra Weiss¹, Niels Holst².

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Collecting data on a continuous basis from bee hives is becoming easier and more common, thanks to improvements in technology and to cheaper electronics. Once the equipment is acquired, the challenge is to find methods of data collection and analysis that extract as much

information as possible from the data. Consider continuous data from hive scales. We have several datasets of hive weight recorded every 15 minutes in different environments and over several seasons. Previously we have fit sine curves to 3 day intervals of averaged

hourly data as a means of measuring foraging activity. While that approach yields robust data on honey bee flight activity, much information is lost via the averaging and the use of multi-day datasets. Recently we tried a new approach. Rather than average data, we calculated the raw daily weight change for each day. Those weight change curves have particular patterns, indicating hive weight changes due to, for example, nectar drying at night, foragers leaving in the morning and returning later, and bees returning to the hive at dusk, as well as other events. We fit a series of straight lines to the data, using a method called piecewise regression (see Figure 11). Piecewise regression provides information on the rate of hive weight change (the slopes of the regression segments) and on the timing of changes in hive behavior (the break points separating the segments). For example, during the active season there is a consistent break point at about dawn as bees leave the hive, causing the rate of hive weight loss to increase compared to the rates during the night. The piecewise regression method provides insight into the behavior of the bee colony during the day and across seasons. We used this piecewise approach to analyze data from a field experiment involving hives that were allowed to forage for pollen compared to hives that were not allowed to forage. While other research approaches had detected no differences in measures such as average individual bee weight, brood or adult bee levels, agrochemical residues or gut microbiota, the piecewise regression method was able to find differences in hive behavior between groups before and after a pollination event. We plan on applying this method to datasets concerning pesticide exposure to honey bees.



▲ **Figure 11.** Within-day hive weight changes. (A) within-day weight change pattern obtained from average 15-minute weight data from 8 hives kept near Madera, CA on 12 April 2014. (B) Same data as above with fitted piecewise regression. Regression parameters are interpreted thus: Point a: Departure of bees at the start of the active period; Point b: Point at which [mass of returning foragers] > [mass of departing foragers + moisture loss]; Point c: Acceleration in hive weight gain, possibly due to fewer foragers departing; Point d: End of forager return and of active period.

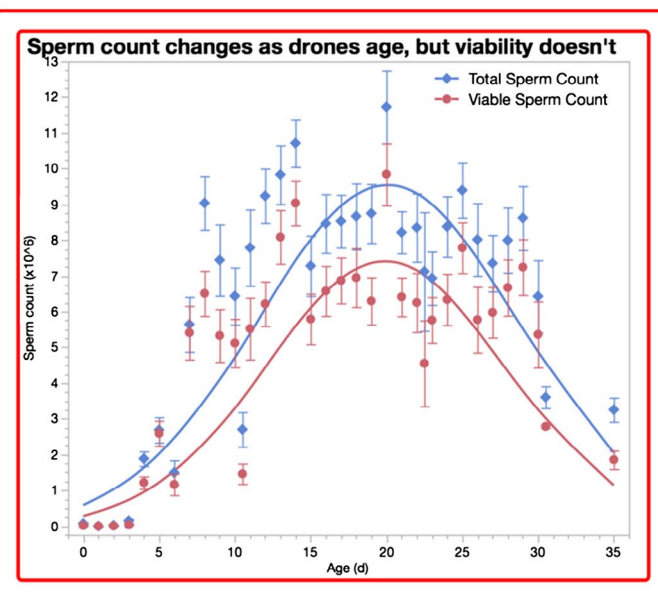
20. Don't downplay drones: Variation in the ontogeny of drone morphological and reproductive quality

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While queen health is critical for colony growth and productivity—and a primary focus for beekeepers in solving management problems—queens are dependent on healthy, reproductively fit drones in order to attain the mating quality necessary to head a highly productive colony. Drones have been historically understudied, and with the observed global decline in male fecundity in many managed animal populations, it is important to question whether perceived declines in queen health are actually a function in decreases of drone reproductive quality. To answer that question, a more complete picture of drone reproductive

variation is necessary, both within and among colonies. We measured the variation in drone body and reproductive development from emergence to 35 d, measuring sperm count and sperm viability in the seminal vesicles, seminal vesicle length, and mucus gland length as measures of reproductive development and body mass, thorax mass, thorax width, wing length, and head width as measures of body development. We found that drones vary widely in all measures. Several reproductive and morphological measures varied significantly with increasing age. Sperm count in the seminal vesicles increased from



▲ **Figure 12.** Picture of a drone used for measurement of head width and thorax width (left) and the relationship between sperm count in the seminal vesicles to drone age (right).

emergence to flight age and then decreased as drones approached 30 d (Figure 12); however, viability did not vary when taking into account the differences in sperm count. Additionally, seminal vesicle length decreased over time and body mass and thorax mass decreased with age. Surprisingly, even measures we predicted to be static (forewing length and head width) also were smaller in older drones. We cannot

currently explain why older drones appear to be smaller, though it is possible that smaller drones develop more slowly and live longer. These findings represent a first step in building the knowledge base necessary to critically evaluate the quality of drones. Our goal is to develop a tool to measure drone fitness potential, measure a colony's ability to produce high-quality drones and the conditions that promote both.

21. The impacts of developmental multi-pesticide pollen and wax exposure on queen honey bee (*Apis mellifera*) health, mating, and colony development

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Honey bee (*Apis mellifera*) queens are challenged by various developmental exposures, which have the potential to not only influence individual queen survival but also downstream colony health (Figure 13). In this study, we reared queens in exposure environments containing realistic pesticide residues and examined the impacts of developmental exposure on queens and their established colonies. We used pesticide treatments consisting of a mixture of insecticides, fungicides and herbicides that have been frequently detected within commercial colonies, and the toxicity of each mixture was based on field relevant Hazard Quotients. We raised queens in simulated exposure environments by grafting worker larvae into queen cells made of treated or untreated beeswax and then placing each into queen cell builder colonies fed a pollen supplement with or without an added pesticide treatment. Following development, we transferred queens into mating “nucs” in order for open mating. After the initiation of egg laying, queens were introduced into standard hive equipment with 2 lbs of adult workers in an artificial swarm. We measured colony growth for three months after

establishment, and at its conclusion we sampled queens for reproductive quality and mating number. We found that colonies that were fed treated pollen had a reduced capacity for queen cell production, and we observed a significant decrease in queen survival during development within constructed queen cells. Queens that developed within cell builder colonies that were fed treated pollen were later found to have reduced sperm counts and sperm viability relative to queens reared within control colonies. Once installed in a full-sized colony these queens were also observed to have reduced brood viability. Our findings suggest that queens that survive development within highly contaminated environments may still be compromised despite having successfully mated. Using a study system that tests oral and contact exposure mixtures developed from previously recorded in-hive residues allows for a new degree of realism in pollinator toxicology. By tracking the downstream implications of our treatments we can better elucidate exposure-associated fitness costs resulting from developmental chemical environments.

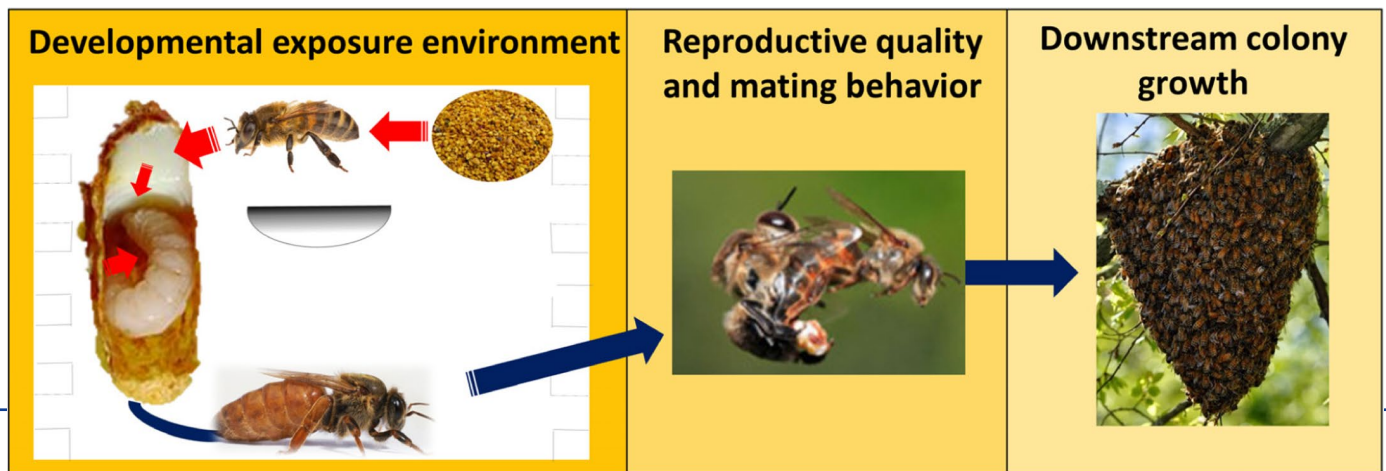


Figure 13. Schematic showing potential downstream effects of developmental queen exposure.

22. Larval dietary stress increases adult susceptibility to clothianidin in honey bees

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Parasites and pathogens, pesticides, inadequate forage, and the culture of beekeeping practices are implicated in worldwide honey bee declines. However, honey bees often encounter combinations of these factors, and the synergisms of these stressors are likely more important in observed declines than any on their own. Neonicotinoid seed treatments migrating from corn fields have previously been linked to reduced overall honey bee health. Here, we evaluate how prolonged pollen deprivation during larval development impacts adult clothianidin susceptibility. Pollen traps were used to deprive half of treatment colonies of this valuable resource during

pollen flows in spring and fall of 2017. The remaining colonies were supplemented with the collected pollen for the duration of larval development (approximately eight days total). Adults subjected to this treatment regimen were emerged in the laboratory and exposed to 0, 10, 40, 200, or 400 ppb clothianidin in sucrose, and mortality was monitored daily. Additional 4th and 5th instar larvae and adults were collected and subjected to total lipids and superoxide dismutase analysis to detect sublethal differences between treatments. Pollen deprived bees experienced greater mortality at sublethal doses of clothianidin compared to pollen supplemented bees, highlighting the importance of larval nutrition in the ability of honey bees to mitigate stress caused by secondary pesticide exposure later in life. The important role of conservation strips in pesticide risk mitigation was discussed.

23. Honey Bee Health Coalition's Bee Nutrition Challenge

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Making a difference can start with one bold idea. That's why the Honey Bee Health Coalition launched the Bee Nutrition Challenge. This effort, launched in 2017, sought creative, practical solutions to accelerate and pioneer the field of honey bee nutrition. Honey bees face serious challenges, partly because they often lack access to a varied and nutritious diet.

Thanks to researchers, we have learned a lot about the nutrients bees need in order to thrive. However, there is a lot we still do not understand about the best diets to keep bees happy and healthy. The Bee Nutrition Challenge sought to address these challenges by seeking out innovative ideas to improve honey bee nutrition. Following a months-long competition, the Coalition identified six finalists out of 24 entries to present their ideas to a panel of judges at the 2018 American Bee Research Conference in a "Shark Tank"-style showdown. The judges then selected four finalists to receive a total of \$40,000 in prize funds and full access to subject matter experts and others from the Coalition's member organizations – including mentoring and advice.

The following teams were awarded money to complete their research over the course of 2018. They will be back to report progress and findings at the 2019 American Bee Research Conference.

- \$15,000 for Miguel Corona, Steven Cook, and Jay Evans, USDA, Agricultural Research Service, Bee Research Lab: *Development and Testing of Optimal Seasonal Nutritional Supplements For Honey Bees*
- \$10,000 for Waled Suliman and Brandon Hopkins, Washington State University: *A Novel Feed Additive for Protecting Bees and Confronting Colony Collapse Disorder*
- \$7,500 for Paul Stamets, Fungi Perfecti LLC: *Fungal Extracts for Honey Bee Health*
- \$7,500 for Jaclyn Nichols, Patrick Heritier-Robbins, Ruchi Banerjee, and Ollie Peterson, Georgia Institute of Technology: *Bee Ultra Sound*

24. Fungal extracts for honey bee health

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Honey bees are known to forage for tree saps and other plant materials that have antimicrobial properties in response to infections. Observation that honey bees forage on wood decay fungi led to speculation that bees may also derive nutritional or medicinal benefit from fungi. Fungi are known to produce a wide array of chemicals with antimicrobial activity, including compounds active against bacteria, other fungi, or viruses. We tested extracts from several species of wood decay fungi (order Polyporales) known to have activity against human or animal viruses for their ability to reduce viral infections in honey bees. In a series of cage trials, extracts from fungal mycelium were tested against two highly prevalent honey bee viruses, deformed wing virus (DWV) and Lake Sinai virus (LSV). It was found that extracts from the

amadou (genus *Fomes*) and reishi (genus *Ganoderma*) groups of fungi significantly reduced virus levels in a dose-dependent manner when mixed into bee sugar syrup feed. Field trials using 5-frame nucleus colonies confirmed the antiviral effects, with treated colonies on average exhibiting a reduction in viral levels to less than 1% the levels found in control colonies that received sugar syrup without extracts. Both fungi significantly reduced both viruses; however, in both cage and field studies amadou extract had a larger effect against DWV and reishi extract had a larger effect against LSV. This suggests that although honey bees may be deriving generalized nutritional value from the extract, they also benefit from specific antiviral compounds in each fungal species that have targeted effects towards specific pathogens.

25. Amitraz exposure increases mortality associated with viral infection in honey bees

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The health and survival of managed honey bee (*Apis mellifera*) colonies are affected by a wide range of factors, one of the most important being the interaction between viral pathogens and infestations of the ectoparasitic mite *Varroa destructor*. Currently, the only effective strategy that exists for minimizing the spread and impact of viral infections is the management of mite infestations, which relies heavily upon the use of apicultural acaricides. Unfortunately, the use of in-hive acaricides comes at a price, as they can produce sublethal effects that are difficult to quantify, but may ultimately be as damaging as the mites they are used to treat. The goal of this study was to investigate the physiological and immunological effects of the formamidin acaricide amitraz and its primary active metabolite in honey bees. Amitraz is a commonly used, beekeeper-applied acaricide, especially in regions where other acaricide chemistries have decreased in effectiveness due to metabolic and target-site resistance in *Varroa* populations. While amitraz does not persist in the hive, its metabolite does, and has been identified as one of the most common contaminants found in the hive environment. Using flock house virus (see Figure 14), a model insect virus system



▲ Figure 14. Infecting honey bees with flock house virus for laboratory assays using a microinjector.

recently described for the study of viral infection dynamics in honey bees, this study found that exposure to amitraz and its metabolite can negatively impact the ability of honey bees to survive viral infection. Furthermore, this work has also demonstrated the disruptive effect that amitraz and its metabolite have on honey bee cardiac physiology, and provided evidence that these cardiac effects are likely due to interaction with octopamine receptors. As this interaction has not

previously been observed in studies at the colony level, these findings stress the need for researchers to do a better job of relating results from individual-level studies of immunity with observations of colony-level effects. More importantly, however, the results of this work suggest a potential drawback to the in-hive use of amitraz and raise intriguing questions about the relationship between insect cardiac function and disease tolerance.

26. Are honey bees feeling antsy? Ants as possible reservoirs of honey bee pathogens

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Despite being a commonly found pest in apiaries, it is not well known how ant species interact with honey bees (*Apis mellifera*) when robbing from or living within their hives (see Figure 15). This is especially true of ant-bee interactions at the pathological level. A single study has looked at this type of interaction and discovered spillover infection of *Deformed wing virus* (DWV) between Argentine ants (*Linepithema humile*) and honey bees (Sébastien et al., 2015 *Biol Lett*). This was the first and only instance to date of the co-introduction and exchange of pathogens between a species of ant and honey bees, but it is not yet known if this is a regional and/or species specific occurrence. The purpose of this study is thus to survey what species of ants are found invading honey bee colonies in Texas and determine if these ant species are serving as reservoirs of honey bee viruses, specifically DWV. Sampling throughout six counties in Central Texas resulted in the collection of six different species of ants found either in or on honey bee hives. This included three Texas native species (*Monomorium minimum*, *Pogonomyrmex baratus*, and *Crematogaster* spp.) and three invasive ant species (*Solenopsis invicta*, *Linepithema humile*, and *Nylanderia fulva*). We conducted diagnostic analyses using PCR in order to screen for the presence of DWV in our collected ant samples. It was found that *Crematogaster* spp. and *Solenopsis invicta* tested positive for DWV. With the use of strand-specific RT-PCR, it was further found that only *Crematogaster* spp. tested positive for active replication of the virus. This study further investigated the understudied concept of cross-species virus transmission involving honey bees.



Figure 15. Fire ants (*Solenopsis invicta*) feeding on a honey bee larva. Photo by A. Payne.

27. Progress on the Canadian Honey Bee Health Survey

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The Canadian National Honey Bee Health Survey is a four-year, nation-wide initiative established to provide an annual snapshot of honey bee health. The survey began in 2014 and will complete its first phase in 2017. The purpose of this project is to document the prevalence, intensity and distribution of pests and pathogens in Canadian apiaries. To accomplish this, brood nest bee samples were collected during the months of July and August, both live and in 70% ethanol, at a sampling intensity of 0.5% of registered hives per region. The survey was designed to systematically expand across the country, starting in Alberta and Manitoba in 2014, and becoming fully national in its third and fourth years. In 2016, 314 composite apiary-level samples, consisting of 10 colonies each, were collected across nine provinces and one territory while in 2017, 255 samples were collected.

Comprehensive information on the distribution and prevalence of 7 major honey bee viruses, *Nosema* spp., *Paenibacillus larvae*, *Melissococcus plutonius*, *Aethina tumida*, *Ascospaera apis*, *Acarapis woodi* and *Varroa destructor* has been compiled using molecular, microbiological and visual detection methods. In addition, samples

were examined for the presence of exotic threats, such as *Tropilaelaps* spp. and *Apis cerana*. These data confirm the endemic nature of specific pathogens, but also indicate where others may still be regionally absent or in low prevalence. Across Canada in 2016, 96.8% of samples tested positive for *Nosema* spp. through molecular detections, with 52.6% infected with *N. ceranae*, 0.3% with *N. apis* and 44.0% co-infected with both species. This distribution was similar in most provinces, with the exception of British Columbia where the only pure *N. apis* infections were found. Using culturing methods, the prevalence of *P. larvae* spores was quantified from samples of adult bees providing a relative risk rating for American foulbrood to individual producers. This culturing also confirmed the presence of oxytetracycline resistance in only two Canadian provinces. In 2016, the survey also incorporated testing for Africanization in Canadian honey bee stock. Surprisingly, 26 apiaries across 5 provinces and 1 territory tested positive for Africanization using current mtDNA testing protocols (PCR-RFLP) while SNP sequencing data for the same samples showed them to range between 0.6 and 15.9% (mean 5.6%) for African ancestry. These results highlight limitations of currently accepted testing methods for detecting hybrids of European and African ancestry.

28. Study of the anatomy of phoretic *Varroa destructor* (Acari: Varroidae) infection via confocal, low-temperature scanning and transmission electron microscopy

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Varroa destructor (Anderson) is the largest single driver of managed European honey bee (*Apis mellifera*) colony losses worldwide. Better understanding of the habits and anatomy of this organism is integral to discovering weak links in its lifecycle potentially leading to sustainable management practices. Recent work has shown that *Varroa* consume fat body tissue as opposed to hemolymph which has long been considered their singular food source. This reframing of our thinking suggests that there is merit in dedicating further attention to the fundamentals of this parasite. In this project, we observed parasitized honey bee and *Varroa* specimens under confocal, transmission and scanning electron microscopy to better understand feeding behavior and the impact of feeding on host tissue. Specimens were stained with toluidine blue and thin sectioned, cryo-preserved, stained with Nile Red and Uranine fluorescent biostains or imaged without advanced preparation. Freeze fractures conducted on cryo-preserved specimens present the first direct evidence that *Varroa* feed on adult bees, showing feeding wounds in the intersegmental membrane after the removal of phoretic mites. Further investigation shows the ultrastructure of these wounds and how they are caused by *Varroa*'s unique gnathosoma structuring.

Investigation of thin sectioned specimens of parasitized honey bees shows the invagination of the intersegmental membrane as the mite's mouthparts extend into it and the eventual penetration and resulting wound. Broken down fat body cells within the wound suggest that the mite feeds via extra-oral digestion as is consistent with the structuring of the mite's digestive system and gnathosoma. Bacterial colonies were observed within the wound itself. Novel techniques developed for this study were shown useful for imaging the structure of soft internal organs of honey bees and mites without dissection. Study via confocal microscopy showed the contents and structuring of the mite's digestive system. Together these methods, both novel and conventional, deepen our understanding of how *Varroa* impact its host on a fundamental level providing details that are difficult to determine via other means. It confirms the conclusion that *Varroa* feed on adult honey bees as has been inferred from indirect evidence in previous study and further provides evidence of the means of acquiring the host meal. These findings contribute valuable insight for the development of chemical and non-chemical control measures.

29. Regulation of mRNAs found post-mating in honey bee (*Apis mellifera*) queen spermathecae

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Because of her specialized function within a honey bee (*Apis mellifera*) colony, the queen's phenotype has been optimized for extreme longevity and reproduction. Our goal is to use molecular tools to identify key genetic markers that help maintain queen longevity. Although we have little information on the mRNAs that are encoded in sperm from insect species, the concentrations of certain mRNAs and miRNAs in mammalian sperm are known to be related to the health and viability of sperm in species, including humans. We used RNA-sequencing to characterize the messenger RNAs (mRNAs) in spermathecae from virgin and mated queens along with semen from drones. Nine cDNA libraries were generated from three spermathecae from virgin queens, three

from mated queens, and three from semen from individual drones. RNA-seq-mediated comparisons identified 13,157 mRNAs across all three tissue types. Ten of the mRNAs were more highly expressed in the spermathecae of mated queens compared to those of virgin queens or drone semen. Using quantitative RT-PCR, we validated the differential expression of those mRNAs across tissue types. We are currently evaluating differential expression of miRNAs in those samples. Our studies shed light on the transcripts that are in honey bee sperm and spermathecae, and will allow us identify the effects of environmental stressors on the expression of these genes, which likely have important functions in the long term sperm storage abilities of queens.

30. Examining the factors influencing *Varroa destructor* host selection of *Apis mellifera* larvae

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Recent declines in honey bee health have been concerning, and the spread of the ectoparasitic mite *Varroa destructor* is thought to be one of the leading causes of these declines. Examining how *V. destructor* finds its larval host for reproduction is important for understanding how it is able to ultimately lead to the death of the colony. Investigating the factors that guide *V. destructor* host seeking is also an important step for developing tools to control *V. destructor* within the *Apis mellifera* hive without the use of toxic acaricides. Here, we investigated the influences of three factors on *Varroa destructor* host selection of *A. mellifera* larvae in two sets of experiments: caste, nurse bee visitation rate, and larval weight.

We found complex interactions among the tested factors. Our comparison among worker and drone cells showed that with increasing nurse bee visitation rates there is an increase chance of cell invasion by *Varroa*. However, drone larvae did not have a higher chance of invasion compared to worker larvae despite higher visitation rates. Worker larvae manipulated through starving and feeding did not exhibit altered nurse bee visitation rates, but did have altered weights. Larvae with increased weight were not shown to have a higher chance of cell invasion. These results give insight in how behavioral factors, such as visitation rate, influence *V. destructor* host selection, potentially overcoming chemical factors in a complex hive environment. More studies like ours are needed to provide essential information on *V. destructor* behavior and improve sustainable methods for the control of this significant bee parasite.

31. Investigating honey bee pollen foraging patterns using multi-locus metabarcoding

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Understanding the floral resources that sustain pollinators is becoming an increasingly popular research theme. Studies investigating floral resource utilization can provide information not only about the plant taxa that bees are reliant upon but also the floral preferences that different pollinating species exhibit. Such information has the potential to inform researchers about the utility of different pollinator species for agricultural pollination, the nutritional quality of forage across different landscapes and the impacts of altered floral resource availability. Here, we employed multi-locus metabarcoding, targeting the *trnL*, *trnH*, *rbcL* and ITS2 markers to characterize the foraging habits of honey bee colonies situated at four apiaries, all within the corn and soybean agroecosystem of central Ohio. A total of 32 pollen samples, collected from May 5th to May 27th, were analyzed to provide a time-series analysis of foraging across the four sampling locations. Further, we conducted microscopic palynology on 12 of the 32 pollen samples (Figure 16) and compared these results with the molecular metabarcoding results. Our data indicate that ITS2 metabarcoding is not quantitatively useful, while analysis of chloroplast markers such as *trnL* and *rbcL* provide accurate characterization of pollen type abundance for mixed-species pollen samples. Further, honey bee colonies forage intensely on a few

key taxa such as pome fruit trees, willows and clovers from mid to late spring in central Ohio.

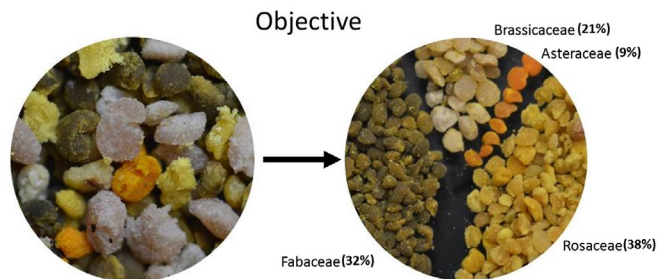


Figure 16. In addition to determining which plant taxa honey bees were foraging on, the objective of this study was to validate the quantitative accuracy of various molecular markers when used in conjunction with DNA metabarcoding or amplicon sequencing.

32. Novel approaches for integrating a compound with poor solubility into honey bee diets for laboratory testing

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Laboratory toxicity bioassays are utilized for assessing the chronic effects of a compound on adult and larval honey bees. Chronic exposure is simulated by integrating a compound into life stage-specific diets, sucrose solution for adults or a royal jelly-based diet for larvae. However, some compounds are difficult to dissolve into these diets and the use of a solvent becomes necessary. Historically, acetone has been used as the solvent for honey bee regulatory testing, but it has toxic effects on its own to larvae, and it can be an ineffective solvent for certain compounds. There is a need for alternative agents for integrating compounds into a diet that can adequately solubilize and disperse. Simultaneously, a potential agent should not have any impact on diet consumption or bee survival. Here we present data on the utilization of sodium lignosulfonate as a potential solvent for use in honey bee

laboratory testing. Our study goals were to estimate relevant toxicity endpoints and to evaluate consumption rates of sodium lignosulfonate in diets provided to honey bee adults and larvae. Bees were exposed to five test levels (0.25, 0.5, 1.0, 2.0, and 5.0%) of sodium lignosulfonate aiming to identify the LC50 (lethal concentration that kills 50% of a test organism) and NOEC (No Observed Effect Concentration). Acetone treatments were included as reference points within the tests (2% acetone). The LC50 and NOEC for adults were 0.99% (95% CI: 0.81–1.17%) and 0.5%, respectively. The LC50 and NOEC for larvae were 1.19% (95% CI: 0.97–1.38%) and 0.5%, respectively. Our research identifies the optimal concentrations of sodium lignosulfonate at less than 0.5% for both the adult and larval honey bee laboratory bioassays.

33. Differential responses to DWV infection in honey bees: A case of tolerance or resistance?

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Honey bees contend with a variety of abiotic and biotic stressors, and this has led to high and likely unsustainable annual colony mortality. The ectoparasitic mite *Varroa destructor* is the biggest threat affecting honey bee health in large part because of the viruses that mites vector while feeding during reproduction and development on honey bee pupae. Deformed wing virus (DWV), in particular, has been noted to be associated with colony losses. Because of the significance of *Varroa*-DWV dynamics, there has been much interest in the relationship between colony mite infestation and viral prevalence. In a few cases, it has been noted that colonies that have natural resistance mechanisms against *Varroa* have lower incidences of DWV infection. However in

other populations mite-resistance seems to be correlated with tolerance to DWV, meaning that mite-resistant colonies survive with high levels of DWV and exhibit fewer symptoms. To clarify whether resistance (maintain low viral titers despite infection) or tolerance (high survival, no symptoms with high virus) to DWV appears to be driving differential effects across honey bee colonies in the absence of mites, pupae from single-drone inseminated queens were injected with a low, moderate or high dose of DWV solution, containing 10⁴, 10⁷, 10¹⁰ viral copies respectively. Pupa showed differential survival and development of the typical DWV symptoms (wrinkled or poorly developed wings, shortened abdomen; see Figure 17) based on colony origin.

A subset of pupae were selected from 5 colonies that displayed low survival and high symptom development at all doses and 5 colonies that displayed high survival and low symptom development at all doses to examine differences in viral titer and antiviral responses via real-time PCR. Overall, at 3 days post-injection, pupae exhibited similar viral titers based on injection dose regardless of colony of origin (e.g., low survival or high survival colonies). The relationship of antiviral responses to DWV level was different from pupae that derived from the low survival colonies as compared to those from the high survival colonies. It appears that honey bee colonies have differential tolerance to DWV infection and that this is at least in part mediated by antiviral responses. However these immune responses do not appear to be sufficient to reduce viral replication, at least at 3 days post-injection. Future work will address the heritability of DWV tolerance in honey bees.



▲ **Figure 17.** Examples of the range of symptom development at emergence resulting from injection of DWV during pupal development. Notice the slight malformation of wings, as evidenced by the wavy wings (first bee on left), to the severely deformed bees exhibiting shortened abdomens and complete lack of wing development (bees on right).

34. Controlled time-release of formic and oxalic acids for *Varroa* control

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Since its introduction to the US in the late 1980s, the *Varroa* mite has been the most serious pest in the beekeeping industry and is a primary cause of honey bee colony loss. Over the last decade, beekeepers in the US have lost a third of their honey bee colonies each year. Mites have developed resistance to earlier synthetic acaricides, and may be developing resistance to Amitraz as well. All synthetic treatments have possible significant limitations, among which are: expensive, labor intensive, unsafe to apply, toxic to developing bees, queen loss, toxic in honey, or build-up in wax. A safe and effective natural treatment for the *Varroa* mite is desperately needed.

Formic acid and oxalic acid are naturally-derived miticides, and resistance to them has not been observed. Formic acid is labeled for use during honey production. Newly registered oxalic acid is not yet labeled for such use in the US, but is effective during the broodless period. Unfortunately, current oxalic acid treatments are “homemade,” resulting in variable doses, and can be hazardous to the applicator. Formic acid, as Mite Away Quick Strips (MAQS), effectively releases formic acid over several days, depending on hive conditions. MAQS cannot be used in much of the country during honey production, because outside temperatures are above those that are safe for use.

MSU has developed a controlled-release formulation for formic acid and oxalic acid that has the potential to be used as an effective miticide in honey bee hives. The strategy involves hyperbranched poly(esters) (HBPE) from “natural”, biodegradable building blocks to which the active ingredient is covalently bonded. As the HBPE degrades, it releases the miticide in a slow, controlled rate. The release rates of these formulations have been studied in numerous laboratory trials. The rate is dependent on temperature and humidity, but the release of formic acid from the HBPE is slower than MAQS under similar conditions, and lasts long enough to kill mites in the brood cycle, not just phoretic mites. The cost of the raw materials is low and the synthetic process is simple and inexpensive.

Most importantly, recent hive tests have been successful. Hives with low initial mite counts (0–1 mites per sugar roll) remained unchanged over nine weeks (still 0–1), while untreated hives from the same batch of bees became heavily infested (40). Other trials showed that hives with significant infestation (17–18) were reduced to zero mite count five weeks after a single dose.

35. The impact of recent honey bee germplasm importations on U.S. commercial bee populations

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Honey bees were introduced into North America by early settlers until further importation was restricted by the U.S. Honey Bee Act of 1922. Although over a half dozen subspecies were imported and released in the US, two main “strains” eventually found favor with U.S. beekeepers: “Italians” derived from *Apis mellifera ligustica* and “Carniolans” derived from *A. m. carnica*. Small founder populations sampled from these original subspecies were propagated to establish the current beekeeping industry. The initial limited sampling of Old World stocks represented a genetic bottleneck for the species (Sheppard, 1989 *Amer. Bee J.* 129: 617–619, 664–666) that was likely exacerbated by loss of feral population diversity and other widespread colony losses attributable to *Varroa* mites.

WSU began importing Old World European honey bee germplasm in 2008 to enhance the genetic diversity of the U.S. honey bee gene pool. We also reintroduced Caucasian honey bees, derived from *A. m. caucasica*, from the Republic of Georgia. The genetic material (semen) is imported under permit by the United States Department of Agriculture (USDA) Animal, Plant Health Inspection Service, assessed for viruses by USDA Agricultural Research Service and inseminated queens are held in an approved quarantine to minimize risk of introducing pathogens.

An important component of this project has been our partnership with queen producers to incorporate the imported stocks into domestic

breeding programs. Queen producers have the ability to mass produce queens for the industry. Surveys of genetic diversity in US breeding populations were conducted before and after the germplasm importations. The impact of these importations has been to enhance the allelic richness (genetic diversity) of our honey bee populations (Delaney et al. 2009, *Ann. Entomol. Soc. Amer.* 102: 666–673). Genetic diversity provides the raw material for selective breeding. Selective breeding represents a sustainable approach to improve the ability of colonies to

resist a wide range of pests and pathogens and adapt to environmental changes.

The imported germplasm collected by WSU since 2012 has also been cryo-preserved in liquid nitrogen, based on recent advances in the technology (Hopkins et al. 2012, *Repro. Fert. & Devel.* 24: 1079). This enables future reconstitution and conservation of subspecies and valued commercial stocks. Using these methods, the USDA has added honey bee germplasm to the National Animal Germplasm Program.

36. Risky business: Honey bee homing behavior during an eclipse

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The ability of honey bees to adapt to new and unique environments is one factor in their success under varying and changing conditions, such as those experienced during migratory beekeeping operations. On 21 August 2017 a total solar eclipse passed across the United States and through Clemson, SC providing a unique opportunity to investigate the behavior of honey bees under novel and risky environmental conditions. While previous studies have shown changes in web building in orb spiders, reduced calling in cicadas, reduced homing in ants, and decreased foraging activity in honey bees during solar eclipses, homing behavior of honey bees has not been studied.

To investigate the effect of the eclipse on the rate of returning bees, we collected data during three time periods on the day of the eclipse (pre-, post- and during the eclipse; total eclipse period was 2 minutes 37 seconds). Older workers and drones from an established colony were marked with fluorescent powder and released approximately 150 m away from their hive. For 40–60 minutes, the number of returning bees at the hive was measured (see Figure 18). Kaplan–Meier statistical tests were used to analyze the rates of return of drones and workers during the three experimental periods.

A comparison of eclipse periods revealed drones returned to the hive more quickly as the eclipse progressed (slowest during pre-eclipse, fastest during post-eclipse); however, workers exhibited a different pattern. While workers also returned most quickly during the post-eclipse period, they returned most slowly during the eclipse, suggesting they are more responsive to the change in environmental conditions than drones. Interestingly, we found that overall, drones returned to hives more quickly than workers. This difference was strongest during the eclipse.



Figure 18. Marked drones and workers were released approximately 150 m away from their hive and every minute, bees returning to the hive were counted. Fluorescent powder allowed the researchers to easily identify the target bees, even when the eclipse reached totality.

This study also provides the first study of homing behavior of honey bees during a total eclipse. Although total eclipses are uncommon phenomena, they provide a unique opportunity to investigate the behavioral flexibility of honey bees. These results show honey bees can quickly adjust to novel and changing environmental conditions, but castes may differ in their responses. This plasticity in behavior is likely an important factor in the success of bees living in inconsistent environments in natural and managed landscapes.

37. A side-by-side comparison of honey bee management systems

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Colony losses are the result of a combination of stressors affecting honey bee health such as pesticide exposure, poor nutrition, parasites, pathogens, and diseases. In response, management for the minimization of beekeeper-associated stressors is a direct way that beekeepers can positively impact colony health. We conducted a side-by-side

comparison of organic and conventional management systems to determine whether either management system results in healthier, less stressed bees. Organic practices involve using cultural control techniques as a first line of defense against hive pests and diseases and avoiding non-organic in-hive chemicals. In addition, these practices

avoid the use of antibiotics, provide treatment for varroa mites only on an as-needed basis, and allow bees to build their own comb (see Figure 19) rather than giving them a comb foundation that could potentially be contaminated with pesticides (Mullin et al., 2010. *PLoS One* 5:e9754). Approximately 98% of beeswax in the United States is contaminated with pesticides that beekeepers, themselves, added to the hive to control varroa mite parasites.

We quantified honey bee health in colonies managed using organic and conventional management practices by measuring honey bee population growth, honey and wax production, overwintering success, varroa mite population growth, and *Nosema apis* and *Nosema ceranae* levels. Because half of the colonies were kept using conventional management and the other half using organic management, we conducted a direct comparison of the impacts of simple management practices as means to keep colonies healthy and both parasite and pest levels low. The results indicate that neither system resulted in a robust, healthy honey bee population.



▲ Figure 19. Example of honey bees drawing comb on frames without being provided the foundation insert.

38. Chemical communication, hygienic behavior, and the development of improved selection tools for *Varroa* control in honey bees

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The health of the honey bee (*Apis mellifera*) is currently being challenged by several natural and anthropogenic threats. Among these are the parasitic mite *Varroa destructor*, and the numerous harmful pathogens it vectors to its honey bee host. While many *Varroa* control methods have been developed, constraints such as limited uptake and the evolution of resistance to some miticides have caused *Varroa* to remain a critical threat to honey bee health. A promising avenue for improved intervention is the breeding of hygienic honey bees, capable of detecting and removing brood that is parasitized or otherwise unhealthy. In an effort to expand our understanding of the role of brood in hygienic

behavior and to improve hygienic selection methods, we investigated honey bee cuticular chemicals associated with unhealthy brood. We identified a chemical that is elevated in unhealthy brood, as well as in brood targeted for hygienic removal. We then demonstrated that this compound and a second, similarly structured compound previously associated with hygienic behavior could induce hygienic uncapping and removal when synthesized and applied to wax caps. These findings expand our understanding of honey bee chemical communication and may lead to improved hygienic selection tools, and thus to honey bees with greater resistance to *Varroa* and its associated pathogens.

39. Honey bee (*Apis mellifera*, L.) queen rearing environment affects behavior and physiology

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Honey bees, beneficial pollinators of vast economic and agricultural importance, have suffered a decline in honey producing colonies for several decades on both national and international scales. This decline is most likely due to many reasons, one of which is pesticide contamination of colony contents. Honey bee colonies collect pesticide residue in sponge-like beeswax comb from their environment or from introduction to colonies by beekeepers. Beekeepers apply pesticides to honey bee colonies to combat pathogens and pests, most notably the parasitic mite *Varroa destructor*.

While lethal effects of miticides have been investigated, there are critical literature gaps regarding sublethal effects of widely used beekeeper-applied miticides. In this study, we found that two historically used miticides which are still in beeswax foundation today (fluvalinate and coumaphos) and amitraz (currently used) cause changes in worker behavior and queen reproductive capabilities when queens are reared in beeswax contaminated with these miticides. Two other common pesticides used in agricultural settings, chlorothalonil and chlorpyrifos, also negatively affect queens when in queen rearing wax.

In this investigation, queens were harvested (see Figure 20) so that mandibular gland contents could be analyzed with gas chromatography. Workers then underwent a choice test of queen mandibular gland contents. Worker retinue behavior to queens was also assessed. Queens reared in pesticide-laden beeswax environments had smaller retinues and lower egg-laying rates than control counterpart queens reared



▲ Figure 20. Picture of an anesthetized queen before dissection. Photograph by E. Walsh

in pesticide-free beeswax environments. Worker retinue behavioral changes may be caused by a change in queen mandibular glands, where the queen mandibular pheromone is produced.

Our results indicate that exposure to pesticides during development alters the reproductive health of honey bee queens by impacting the queen pheromones, which are what queens use to attract drones

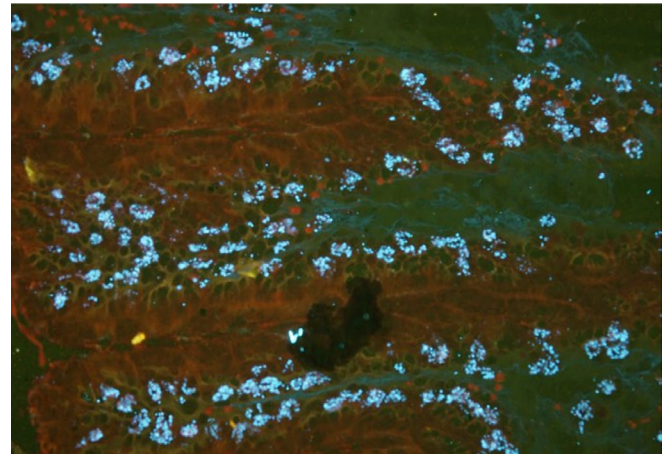
during mating flights and attract queen attendant caretakers. Our results have important implications regarding the potential impacts of beekeeper-applied miticides and agrochemicals on colony health. In light of our findings, it is clear the beekeeping industry needs to adopt an integrated pest management approach to *Varroa* control where cultural and physical-mechanical methods of control are utilized before pesticides.

40. Early indicators of *Nosema ceranae* infection in honey bees

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Early indicators of *Nosema ceranae* infection are helpful because they can allow controlled tests of potential treatments for the disease, or breeding stock, to be done more quickly. Also, the source of infection might be identified. For example, package bees with well-established infection must have acquired the disease more than (for example) 8 days earlier. Bees with newly established infections must have acquired them relatively recently. We examined worker bee midguts by histological sectioning and selected fluorescent stains. Alizarin red and calcofluor white clearly show discrete initial infection loci within some midgut epithelial cells (see Figure 21) beginning at 9 days post-inoculation (9 dpi). This suggests that each cluster of developed spores corresponds to one site at which a spore initiated an infection. We expected to see infection sites primarily at the tips of the epithelial folds which protrude toward the lumen. It seemed that these protruding folds would most easily be reached by the polar tubes of germinating spores. To our surprise however, infection sites were equally abundant in the invaginations of the epithelium farthest from the midgut lumen. At 10 dpi the infections were more diffuse and contiguous, but had not yet spread to all epithelial cells. By 14 dpi, the entire midgut was infected, including the cells adjacent to the basement membrane.



▲ **Figure 21.** Example of early infection of midgut epithelial cells by *Nosema ceranae*.

41. Diamide modulation of muscle fatigue in honey bees

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The loss of honey bee (*Apis mellifera*) colony numbers in recent years presents an economic and ecological impact on agricultural practices and services provided by these pollinators. One outstanding threat is the unintended exposure of these pollinators to agrochemical stressors. Diamide insecticides are a relatively new mode of action on the agrochemical market. These insecticides modulate the ryanodine receptor, a novel calcium ion channel target site in insect muscle cells. These chemistries have low vertebrate toxicity, minimal environmental persistence, and high selectivity for pest species over beneficial insect species.

There are relatively few studies that report the potential for diamide insecticides to negatively affect pollinator health. The aim of this study focused on the toxicological, biochemical, and behavioral effects of diamide insecticides to honey bees. We reported the acute toxicity, metabolic detoxification activity, and behavioral fatigue of honey bees exposed to three diamide insecticide formulations, as well as discussed continuing research on a mechanistic approach to better understanding the effects of diamide insecticide exposures to these pollinators.

42. Evaluating the abundance and diversity of pollinator communities in enhanced and natural turfgrass habitats

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Honey bee losses and declines in health and productivity are often associated with a number of factors, including a lack of high quality forage. Land conversion is believed to play a role in the decline of forage availability, as natural areas like prairielands and woodland forests are being converted to agricultural areas and urban metropolises to meet the needs of a growing human population. Because of this, as well as the rise in the number of beekeepers in urban areas, it is essential to start considering bee conservation within the confines of an urban setting. One potential route for improving bee health within urban areas is through habitat enhancement in turfgrass areas. Turf lawns are

typically managed as a highly managed mixture or blend of different turfgrass species to maintain a desired aesthetic, or recreational function. The flowers that occur within these areas are typically viewed as weeds, dismissing the potential value that these flowers hold for bees. The flowers that occur within these turf lawns have the potential to provide forage for honey bees and other bee pollinators. Additionally, if these turfgrass areas are redesigned such that low growing flowers are intentionally introduced to provide additional forage for bees, it is possible to maintain the aesthetic and recreational function of turf lawns, while also repurposing these areas for bee conservation. In

this study we seek to compare the abundance and diversity of bees within parks with naturally occurring white clover, and parks that have been enhanced with low growing forbs. Four Minneapolis public parks within Minneapolis were selected for enhancement, with each enhanced park paired with a natural, or unenhanced park, nearby. Twenty minute transect walks were conducted once per week where

bees were collected off of flowers with a bee vacuum. Specimen were then pinned and identified to the species level. We compared the abundance and diversity of bees at each park to determine the relative value of enhanced and unenhanced parks. This study provides insight for future studies examining the potential for habitat management practices within urban settings.

43. Entomological Society of America's Plant-Insect Ecosystem Section (ESA-P-IE) science policy field tour: Bringing together diverse stakeholders to foster communication

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The Entomological Society of America's (ESA) Plant-Insect Ecosystems Section (www.entsoc.org) organized a Science Policy Field Tour: *Balancing Pest Management and Pollinator Health* in cooperation with Mississippi State University on 22–24 August 2017 in the Mississippi Delta. The event brought together ESA members and key stakeholders representing 22 states and the District of Columbia. Stakeholder groups included representatives from federal and state public science agencies, policymakers, NGOs, beekeeping organizations, and crop protection and commodity groups. The field tour enabled hands-on understanding of pollinators and the many issues on which they intersect, including: pollinator habitat, pests of pollinators, beekeeping practices, insect pests of economic importance, and row crop production and management.

The field tour enabled candid discussions with the goal of learning from the experiences of Mississippi stakeholders that were instrumental in the development of the Mississippi Honey Bee Stewardship Program (MHBSP), one of the first programs developed nationally. The tour concluded with a discussion among participants about how to continue sharing ideas and best practices as other pollinator protection efforts are developed. Several themes emerged from the tour about the MHBSP and included: the need to build relationships, invite all interested parties to the table, stay at the table, build awareness, use integrated pest management and remain flexible. This event led by ESA provides a model for how a professional society can serve as an organizational leader to create an unbiased context for an event and take an active advocacy role when work done by scientists impacts issues affecting the public.

Abstracts of Poster Presentations

44. Using 'omics' tools to breed better Canadian bees

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Overwintering mortality of honey bee colonies in Canada has been continuously greater than the acceptable range of 0% to 15% since the winter of 2006/2007. The main causes of colony death, as reported by Canadian beekeepers, include high pathogen/parasite infestation levels (e.g., *Varroa* mites, *Nosema* spp.), poor quality queens and severe weather conditions. Every year, Canadian beekeepers import hundreds of thousands of queens, mainly from the U.S.A. and New Zealand. The importation of foreign queens has the potential to introduce undesirable pathogens or genetics and supply bees that have not been selected to survive in northern temperate climates. The goal of our project is to measure 12 economically-valuable traits of honey bees (colony phenotypes) and develop genomic and proteomic markers for each trait that will enable beekeepers to rapidly select and breed healthy and productive colonies that are well adapted to the Canadian climate. In 2016, 1025 colonies across Canada (British Columbia, Alberta, Manitoba, Ontario and Quebec) were sampled and phenotypic data was collected for the following colony-level traits: 1) *Varroa* mite population growth 2–4) grooming, hygienic and defensive behaviour; 5) honey production; 6) sealed brood population; 7–9) pathogen abundance (viruses, *Nosema* spp., Trypanosomatids); 10) innate immunity factors; 11) gut microbiota; and 12) overwintering success. The identification of bio-markers for each trait, and the variation of each trait among

colonies located in different landscapes and climates in Canada, as well as the correlation between phenotypes comprised the first step of this novel research. To date, we have performed multiple correlation analysis to study the relationship among all traits. The two highest correlation values found are from between fall and spring total gross colony weights, and total and instantaneous honey production (see Figure 22). Colony weight in the spring (after winter) is highly correlated with its weight in the fall (before winter; $R^2 = 0.8374$; $P < 0.001$). We also found that total honey production is highly correlated with total colony weight gain during 2 weeks of peak honey flow ($R^2 = 0.6905$; $P < 0.001$). This result is supported by previous research from T. Szabo who found that 86% of the net honey production occurs in 14 days during the peak of honey flow (Szabo T and Heikel D, 1987 *J. Apic. Res.*, 26(1): 47–52). In the summer of 2017, 496 colonies were sampled and putative markers were validated against a test population, with the end goal of having this technology transferred to end-users, such as the National Bee Diagnostic Centre (Beaverlodge, AB), where it will be made available to beekeepers. This is the first large-scale study for marker assisted selection in honey bees using integrated genomics and proteomics tools. Our innovative research will promote a healthier honey bee population and support the sustainability of the Canadian beekeeping industry.

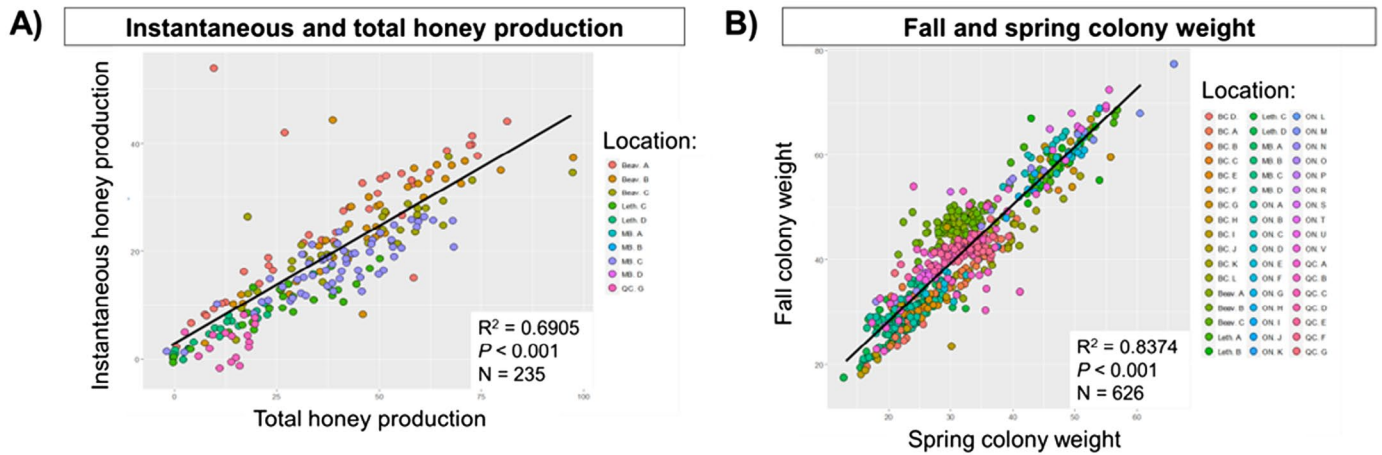


Figure 22. Correlation between (A) instantaneous and total honey production and (B) fall and spring colony weights.

45. Blocks for bees: Solving bee business problems with blockchain technology

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There are many threats facing bees today. Some of these threats are biological and some are business threats. Technology has a role to play in helping alleviate some of the threats facing bees and beekeepers, both from a biological and business standpoint. In particular we discussed the emerging role that Blockchain, also called “distributed ledger,” technologies can serve in helping beekeepers manage their business better (Figure 23). Specifically we explore the three business concerns of honey adulteration, smart contracting for pollination services, and insurance pricing and claims verification. We explained the underlying technology and discuss how it can provide integrity and efficiency in transactions.

First, we described how these features have the potential to reduce honey adulteration by providing for secure production and supply tracking. This product tracking allows honey producers to make provable assertions about the quality of honey products to retailers and consumers. We also illustrated how smart contracts based on the blockchain can help streamline the pollination contracting process. This is done by creating an automatic fulfillment system that cannot be forged. Finally, we demonstrated how this technology could help with hive insurance and claim verification. This is done by using the security of the data to prove insurance claims are not fraudulent and that the beekeeper fulfilled all their insurance obligations.

These business model improvements are promising because they have the potential to bring many beekeeping practices into contact with the computer and data driven technological world in new ways beyond what has been possible or practical in the past. Our model creates a framework for providing incentives to beekeepers to keep data and lays the groundwork for easier analytical work in the future to improve

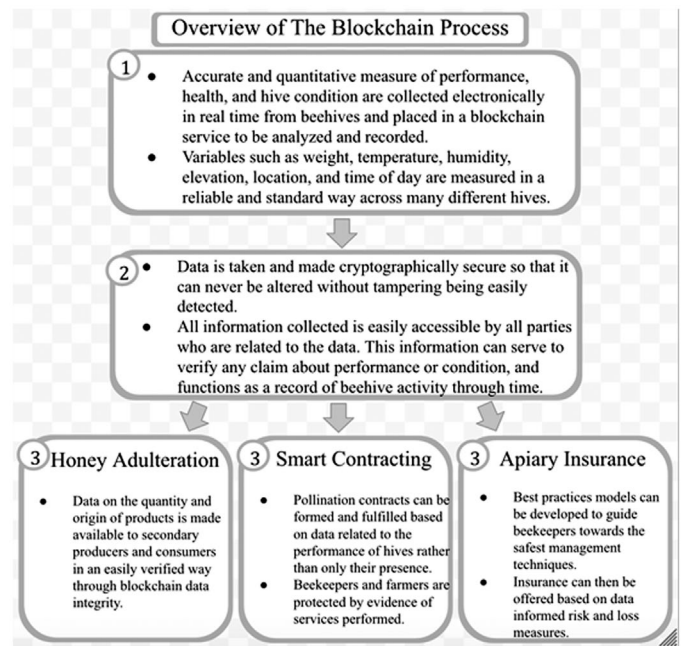


Figure 23. Flow chart describing how Blockchain process can facilitate business improvements for the beekeeping industry.

the beekeeping industry in honey production, bee health and survival, and pollination, while also opening the door to financial safeguarding through agricultural insurance.

46. The effects of the insect growth regulators methoxyfenozide and pyriproxyfen and the acaricide bifenthrin on honey bee (*Hymenoptera: Apidae*) forager survival

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The honey bee (*Apis mellifera* L.) contributes an essential role in the United States economy by pollinating major agricultural crops. Among the top benefactors of this ecosystem service is almond, which depends entirely on honey bee pollination for successful nut set. Similar to other agricultural systems, almond orchards are challenged by a variety of pests and pathogens that inhibit crop productivity. Such threats are frequently managed with a variety of agrochemicals, including insecticides, which are applied to control pests during bloom. While the effects to honey bee health of some insecticides, particularly neonicotinoids, have received attention recently, the impact of other types of insecticides on honey bee health is less clear. In this study, we examined the effects to honey bee forager survival of three non-neonicotinoid pesticides widely used during the 2014 California almond bloom.

We collected foragers from a local apiary and exposed them to three pesticides at the label dose, or at doses ranging from 0.5 to 3 times the label dose rate. The selected pesticides included the insect growth regulators methoxyfenozide and pyriproxyfen, and the acaricide bifenthrin. We simulated field exposure of honey bees to these pesticides during aerial application in almond orchards by using a wind tunnel and atomizer set up with a wind speed of 2.9 m/s. Experimental groups consisting of 30–40 foragers each were exposed to either untreated controls or pesticide-laden treatments and were monitored every 24 h over a 10-day period. Our results revealed a significant negative effect of all pesticides tested on forager survival. Therefore we suggest increased caution in the application of these pesticides in almond orchards or any agricultural crop during bloom to avoid colony health problems.

47. The vital role of hive management in honey bee tier II studies

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To create a valid and study-ready colony for Tier II colony effect studies, there are numerous factors to consider that influence the stability, longevity, and fecundity of a honey bee hive. One must understand, and implement both broad and subjective colony management practices, namely an intimate understanding of honey bee physiology, including queen bee and drone viability, pest and disease management, hive economics, resource needs and allocation, division of labor, and the environment and its effects on each of the aforementioned. Not only does a beekeeper have to understand these core concepts, they must also be able to implement them on a hive to hive basis, providing specialized care and maintenance of each hive.

Hive management practices and hive history are as important to producing quality data as proper study conduct and adherence to GLP standards. Tier II Studies, with their many limitations, present additional challenges and yield confounding, misleading, or specious results. Therefore, bridging the gap between beekeeping and honey bee ecotoxicology studies is integral to ensuring a quality data set, and efforts have been made to focus on preparation and vetting of honey

bee colonies as a test system. The vetting process includes a myriad of metrics including nutritional assessments and tailored supplementation of all colonies, pest/disease management to optimize healthy generations of bees prior to study initiation, and queen fecundity and brood termination rate assessments of honey bee colonies to aid in colony selection for Tier II studies. These measures give us the ability to judge uniformity and validity of naturally occurring brood termination rates for candidate colonies via larval termination assessment in the field, which directly correlates to an increase in study acceptability and interpretability. Additionally, through hive propagation, we are able to provide sister queens for Tier II studies, resulting in data that allows for more comparative analysis by decreasing genetic variables inherent in the study. Best beekeeping practices preceding and during a study; including vetting and selecting test system colonies, has yielded positive results. In OECD75 effect studies (see Figure 24), these practices yielded brood termination rates within range of study acceptability. In yearlong feeding studies, best beekeeping practices yielded year after year overwintering losses at 12.5% for untreated control colonies. Best beekeeping practices and knowledge of hive history enables quality bees for quality data.



Figure 24. OECD75 effect study tunnel design, inside tunnel, brood photo; optimal brood development. Photography by Eurofins Agrosience Services Bees Health and Management Solutions.

48. Using logistic regression, k-nearest neighbor, and support vector machines to classify audio samples in audio bee-hive monitoring

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Electronic beehive monitoring extracts critical information on colony behavior and health without invasive beehive inspections and reduces transportation costs through wireless data transfer. Remote audio monitoring helps distinguish dead from live hives and thriving from failing hives. BeePi is a multi-sensor electronic beehive monitor that consists of a raspberry pi computer, a camera, a temperature sensor, a clock, and three microphones embedded in hive walls. All BeePi components are off-the-shelf and fit in a shallow Langstroth super. BeePi was piloted in 2014. In 2015, two BeePi units were tested in Northern Utah in two Langstroth beehives for two weeks. In 2016, four BeePi units were tested in Northern Utah for two months. In 2017, four BeePi units were deployed in Northern Utah for five months (May to September) to collect 150GB of video, audio, and temperature data. Three machine learning methods, logistic regression, k-nearest neighbor (KNN), and support vector machines (SVM), were tested to classify BeePi audio samples as honey bee buzzing, cricket chirping, and ambient noise. A sample of 260 30-second audio files captured by deployed BeePi monitors was taken. Each audio was segmented into 14 2-second audio samples. Each 2-second audio sample was manually labeled as honey

bee buzzing, cricket chirping, or ambient noise. A sample of 3,300 labeled samples (1,100 bee buzzing; 1,100 cricket chirping; 1,100 noise) was used for training and testing with 60% used for training and 40% for testing. Each 2-second audio sample was turned into a vector of 193 features: 40 mel frequency cepstral coefficients, 12 chroma short term fourier transform coefficients, 128 melspectrogram coefficients, 7 spectral contrast coefficients, and 6 tonnetz coefficients. All testing was done with the scikit-learn package of Python 2.7 on an Intel i7 PC with 15GiB of RAM. Each model was tested with k-fold cross validation ($k = 10$). Logistic regression achieved the best accuracy of 99.75% with standard feature scaling. In testing KNN, the number of nearest neighbors, K , varied from 1 to 25. The best accuracy of 99.79% was achieved with $K = 1$ and uniform feature scaling. The SVM was trained using the one-vs-the-rest classification, e.g., bee vs. cricket or noise. The best SVM mean accuracy of 99.69% was achieved with min max feature scaling. The results show that all three machine learning methods perform on par. The results also indicate that standard machine learning methods have potential in remote audio monitoring of beehives.

49. Using overwintered nucleus colonies for early spring research

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Traditionally, nucleus colonies are small hives with five frames of brood, bees and a laying queen. They are often available for sale during the spring months, along with honey bee packages. Beekeepers use them to expand their apiaries or to replace winter losses. Michael Palmer has been keeping nucleus colonies in an alternative method in Saint Albans, Vermont. The nucleus colonies are initially established during the spring honey flows (see Figure 25), and then expanded to grow vertically by adding an additional box. The colonies are managed prior to the winter. 240 nucleus colonies were made in Clinton Co. New York during June and July of 2016. Each colony was started with two frames of brood and a queen cell. They were grown out to 10 frames or 5x5 configuration. 127 colonies were treated, equalized and moved to Columbus Co. North Carolina. In January 2017, 48 colonies were used for a migratory study where colonies were sent from the coast of North Carolina to California almonds. We believe overwintered nucleus colonies are ideal for early spring research. All colonies met minimum contract requirements for the almond broker. Colonies had a high level of standardization amongst the group. Queen quality and overall colony health was well observed. Outliers were omitted from selection for the experiment. Instead of purchasing packages or nucleus colonies in the spring, this project exemplifies how Palmer style management of nucs can be used to establish quality colonies for research efficiently and cost effectively.



▲ **Figure 25.** An overwintered nucleus colony early the following spring. The colonies were treated, fed and equalized prior to the winter. The queens establish a large population early, making them excellent choices for early spring research.

50. Longitudinal evaluation of supplemental forage on honey bee colonies in California almond orchards

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Access to plentiful and diverse forage helps honey bees better deal with stressors, including pathogens (DiPasquale et al. 2013, *Plos One* 2013, 8(8):e72016) and pesticides (Schmehl et al. 2014, *J. Insect. Physiol.*, 71: 177–190). Managed honey bees are used for pollination for dozens of crops in California. Almonds are the first crop, requiring approximately two million strong colonies in February. While almonds can supply plentiful

nectar and pollen, there are minimal, naturally occurring options for additional forage at this time. This has prompted calls for supplemental forage plantings in areas where colonies are present during the pollination and honey production cycle. The period before, during and after almond bloom perhaps is the greatest opportunity to provide supplemental forage that could benefit the most colonies at once. Our main interest, within the

context of a larger collaborative project initiated by Dr Neal Williams (UC Davis), is to evaluate the immediate and long-term benefits of two different supplemental forage plantings in almond orchards during pollination, on honey bee colony health, growth and survival.

During the 2017 almond bloom we evaluated colonies provided access to mustard plantings (supplied by Project Apis m) or wildflower plantings (see Lundin et al. 2017, *Environ. Entomol.* 46(3): 559–564) and appropriate almond-only controls (see Figure 26). We recorded the size of adult bee population, brood production, pollen and nectar/honey stores, and varroa mite levels, immediately before, twice during, and once post almond bloom. Pollen was also collected from colonies at several time points and plant species were identified (Dr N. Williams Lab). Bee samples were collected for further pathogen and immune gene analyses (samples being processed by Dr Q. McFrederick, UC Riverside and Dr K. Anderson, USDA-ARS, Tucson, AZ).

Unusually cold and wet weather precluded wildflower plantings from reaching full bloom during the time that the bees were in orchards although after the almond bloom we were able to identify that a portion of pollen collected was indeed from wildflowers. Bees collected significant proportion of mustard pollen in the mustard-planted areas (Cibotti, Ward, Williams, personal communication). We ultimately compared mustard and matching control colonies only. Adult bee population size as well as brood amount were significantly greater in mustard-supported colonies as compared to almond orchard controls, at two time points during almond bloom ($P < 0.05$). Once colonies were transferred to a common apiary, potential colony growth benefits were lost indicating the importance of continued access to diverse forage. We did not find any forage effects on mite infestation. However, we would expect pathogen and immunity analyses to be more robust and potentially more likely to reveal forage benefits. Colonies from mustard-supplemented orchards had the greatest, but not significantly so



Figure 26. Experimental colonies were evaluated for various colony metrics and were outfitted with pollen traps for pollen collection and future pollen analysis.

Photo by E. L. Niño Bee Lab.

survival when the colonies were inspected several months after almond bloom. Overwintering colony survival will be determined in 2018.

Our preliminary analyses support the widely-held inference that access to supplemental forage during almond bloom could have benefits for colony growth and likely bee health and survival. It also highlights the importance of having continued access to plentiful and diverse forage throughout the year.

We thank the Almond Board of California (16-POLL20-Nino) and California State Beekeepers Association for funding this work.

51. The *Tropilaelaps* mites threat: An examination of the injuries inflicted on *Apis mellifera*

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Tropilaelaps spp. are the most serious parasites of *Apis mellifera* in Asia. However, much of the biology and ecology of this parasite is largely unexplored (de Guzman et al., 2017 *J. Econ. Entomol.* 1–14). Like varroa mites, tropilaelaps mites puncture through the integuments of their bee hosts to feed on hemolymph. In this study, we examined the types of injury inflicted by *T. mercedesae* on different stages of *A. mellifera* in Northern Thailand. We report here for the first time that

these mites feed on unsealed larval stages of honey bees. *Tropilaelaps* mites are reported to have short survival outside capped brood cells (Rinderer et al., 1994 *J. Apic. Res.* 33: 171–174). Thus, this ability of tropilaelaps mites to feed on unsealed brood may increase their survival when no suitable host for infestation is available, and may also contribute to their successful reproduction. Unlike varroa mites that inflict 1–2 large wounds (Kanbar & Engels, 2004 *Apidologie* 35: 25–29),

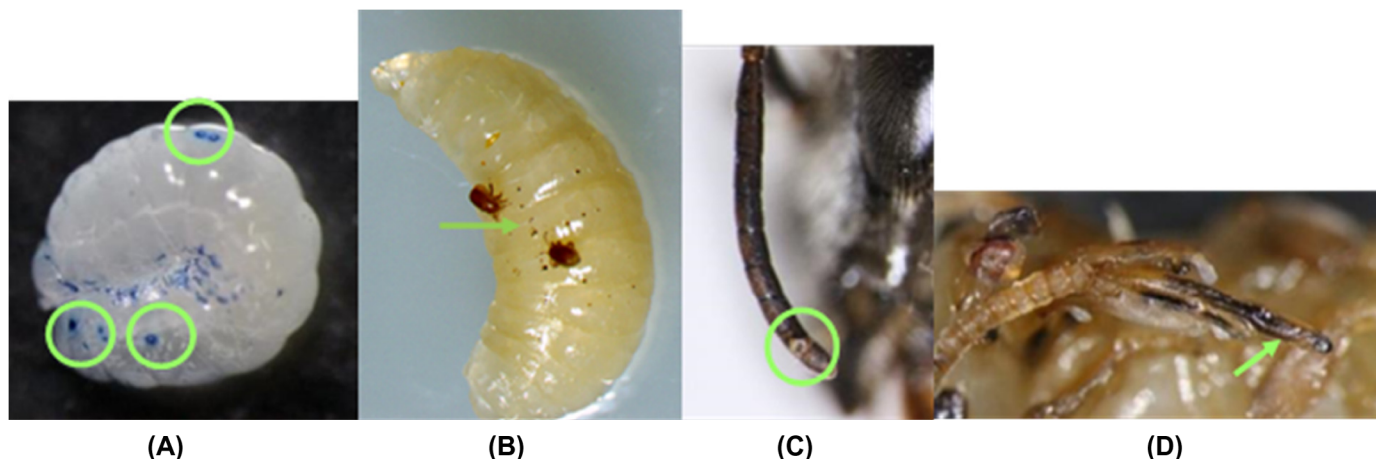


Figure 27. Damages inflicted by *Tropilaelaps mercedesae* on honey bee hosts: (a) multiple wounds on 2nd instar larva (L2), (b) multiple wounds (brown to black scars) on 6th instar larva (L6), (c) a hole on an antenna, and (d) darkened proboscis.

tropilaelaps mites cause multiple wounds especially on larval stages (see Figure 27(a, b)). Tropilaelaps mites are vectors of deformed wing virus (Khongphinitbunjong et al., 2015 *J. Apic. Res.* 54: 40–47). Hence, transmission of pathogens and secondary infections may commence early during the bee's life, which may contribute to shortened lifespan

of adult bees. In addition to wing deformation, injuries were observed on different parts of the bees' body including the mouthparts, legs, abdomen, thorax and antennae (see Figure 27(c, d)), which may significantly accelerate death or removal of these injured bees from the colonies by house bees.

52. Neglected problems in beekeeping and beekeeping best management practices

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Invasive species usually have an adverse impact in agriculture. The arrival of invasive species challenges current ecological views and our accepted management practices. This creates the need for innovative knowledge concerning the biology of the invader and their effects on current biodiversity. Invasive pests of the honey bee hive, such as the small hive beetle (*Aethina tumida* Murray (Coleoptera: Nitidulidae)) and the Varroa mite (*Varroa destructor* (Anderson and Trueman (Acarina: Varroidae))), cause bees to abandon the hive and colonies to fail. They leaving behind pollen, honey, wax and dead bees, which eventually become a food source for other organisms to feed upon (see Figure 28). Two insect species are emerging as opportunists that can present problems in beekeeping. For example, the Dermestidae beetle that feeds on dead adult bees and immature stages abandoned inside the brood cells (See Figure 29). Another example is the Indian meal

moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) that feeds on pollen stored in honey bee frames (See Figure 30). We have observed both pests as well as their impacts in the colony and on stored equipment. Although primarily scavengers, these opportunists can become nuisance pests by destroying valuable beekeeping equipment (e.g., wax combs) or depleting resources acquired by the bees (e.g., stored pollen) leading to economic losses for the beekeeper. These pests can be managed using sanitary actions aimed at reducing infestation and avoiding their spread to new locations. Fortunately, such sanitary actions are compatible with established controls for wax moths and other well-known threats to honey bee colonies. Other arthropods such as ants, predators and parasitoids also impact beekeepers through damaged equipment, use of food resources, and possibly disease transmission. Awareness of these neglected pests can lead to better beekeeping methods and a reduction of their impacts.



Figure 28. Remains of dead colonies and opportunistic pests. (A) Frame with dead brood, (B) frame with moldy pollen, (C) frame with honey and dead brood, (D) pile of frames for cleaning or trashing, (E) pieces of comb scraped when cleaning frames, (F) Indian meal moth and Dermestidae beetle found attached to sticky board placed in storage room.



Figure 30. Biological stages and damage caused by Indian meal moth (IMM). (A) adult, (B) adult laying eggs, (C) egg and a first instar larva, (D) web made with silk to hide, (E) cell sealed with silk and a deposit of feces, (F) larva inside the cell, (G) larvae and web of silk, (H) pupae (I) pollen infested with IMM, (J) parasitoid *Apanteles* spp., (K) parasitoid *Casinaria* spp., (L) sticky board.

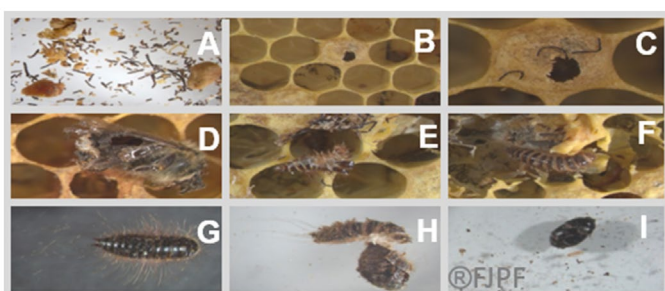


Figure 29. Biological stages and damage caused by the Dermestidae beetle. A) excrement of the Dermestidae beetle, B), C), cells with dead brood showing signs of infestation by the Dermestidae beetle, D) Dead bee eaten out by the beetle larvae, E) Exuviae of larva, F), G) larva, H) pupa, I) adult beetle.

53. Gluconic acid may inhibit *Nosema ceranae* infection in honey bees

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Gluconic acid, one of the constituents of honey, was found to inhibit the development of *Nosema ceranae* in caged worker honey bees. A preliminary experiment suggested that 0.5% gluconic acid in 50% sucrose syrup was more effective in reducing infections than higher or lower concentrations. This happens to be the approximate naturally occurring concentration of gluconic acid in honey. In Trial 1, worker bees were individually fed 20,000 *N. ceranae* spores in 50% sucrose, and then caged collectively and kept in an incubator at 32 °C for 10 days. Half of the caged bees were fed 0.5% gluconic acid in 50% sucrose and half were fed 50% sucrose without gluconic acid for the 10 days.

Those treated with gluconic acid were found to contain no spores after the 10-day period, while the control bees contained a mean of 7.6 million spores per bee. For trial 2, worker bees were inoculated in the same way. Then half of the bees were fed 50% sucrose for 2 days, and followed by 50% sucrose with 0.5% gluconic acid for the remaining 8 days. The control bees received only 50% sucrose. In this trial, the bees developed a mean of 19.4 million spores per bee by day 10 for control bees. Those receiving gluconic acid after day 2 developed a mean of 16.2 million spores per bee. This was not a statistically significant difference. We plan more extensive studies in the coming year.

54. Effective protections and environmental factors have strong impacts on the composition and diversity of the gut bacterial community of Chinese black honey bees

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The Chinese black honey bees, an ecotype of the European honey bee that is formed by natural hybridization of *Apis mellifera mellifera* and *A. m. carnica*., has been an important breeding resource for disease resistance and other desirable traits. In the previous studies on Chinese black honey bees, little attention has been paid to the diverse population of gut microbes that play a vital role in host health. In the present study, we analyzed the gut bacterial communities of Chinese black honey bees using terminal restriction fragment length polymorphism (T-RFLP). The results showed that the samples from the national nature reserves that are protected and managed so as to preserve and enrich their natural condition and resources for Chinese black honey bees had higher variety and richness of gut bacteria than that collected from unreserved regions that also harbor populations of Chinese black

honey bees. The four terminal restriction fragments (T-RFs) 201, 223, 247 and 320 bp were inferred to be the beneficial symbiotic bacteria according to previous study. Of which 247 and 320 bp had greater differences between sample groups and could be used to separate samples collected from the national nature reserves from samples collected from unreserved regions. The results indicate that the national nature reserve protects biological diversity and ecological and evolutionary processes which have had significant influence on the diversity of gut bacteria of Chinese black honey bees. The ubiquity of gut symbiotic bacteria identified in Chinese black honey bee suggests that environmental factors could play an important role in diversity and composition of gut bacteria and warrant further investigation into the functional significance of these gut bacteria for the honey bee health.

The Asian hornet - threats, biology and expansion

The accidental introduction of the Asian hornet (or more accurately the yellow-legged hornet *Vespa velutina*) into France and South Korea over ten years ago, and its subsequent spread to neighbouring countries has been worrying to both governments and beekeepers alike. Many people are now seeking more information on this new threat to our beloved honey bees. Hornet biology is very different to that of honey bees and this can lead to misunderstandings and false assumptions. So, this book written by Prof. Stephen Martin of Salford University, UK, is aimed at anyone wanting to learn more about hornets, why the Asian hornet possesses such a threat, and what can be done about it.



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