A Long-Term Plan to Improve Honey Bee Genetics: Formation of a Tech Transfer Team

Project No.: **10-POLL5-Spivak**

Project Leader: Dr. Marla Spivak
University of Minnesota
1980 Folwell Ave.
St. Paul, MN 55119
(612) 624-4798
spiva001@umn.edu

Project Cooperators and Personnel:
Susan Cobey, UC Davis
Drs. Walter Sheppard and Timothy Lawrence, WSU-Pullman
Katie Lee, UCCE - Butte County

Objectives:

The specific objective of this proposal was to obtain one-year, partial salary support for a professional to lead a Tech-Transfer Bee Team to assist honey bee breeders based in Northern California. The Bee Team will assist commercial queen bee rearing operations with

1. Stock selection and breeding for resistance traits
2. Enhancement of genetic diversity in bee stocks
3. Perform disease and pest diagnostics and provide information on integrated management practices to reduce chemical treatments
4. Facilitate cooperative research on relevant topics (e.g., quality of queen and drone bees; nutrition)

The long-term objective is to establish a permanent, fiscally self-sustaining Bee Team consisting of 2-3 professionals and serve as a model for the establishment of other Bee Teams throughout the country.

Interpretive Summary:

Katie Lee (UC Cooperative Extension, Butte County) was hired to initiate the Bee Team. She organized and conducted stock selection and disease / pest diagnostics for 15 bee breeding operations from December 2010 through February 2011, prior to the beginning of queen producing season (end of February). A 16th operation was sampled in May. Each of the 16 participating queen producers paid a $500 fee-for-service to fund supplies and truck rental.

Queen producers were asked to select colonies from their own stocks of bees that had potential as breeder colonies or choose an apiary with exceptional colonies. Ms. Lee diagnosed those colonies for the parasitic mite *Varroa destructor* and the gut fungus *Nosema*, and tested for hygienic behavior. Colonies with hygienic behavior have natural resistance to

brood diseases and the *Varroa* mite, while still being productive and gentle (Spivak and Reuter, 1998). The intent was for the bee breeders to choose the best performing colonies with the lowest levels of *Varroa* and *Nosema*, and the highest levels of hygienic behavior. A second round of disease and pest diagnostics was conducted for 12 of the queen producers in May-June to provide *Varroa* and *Nosema* levels to help them make informed treatment decisions. Overall, Lee collected and analyzed 829 samples for *Varroa*, 1049 samples for *Nosema*, and tested 558 colonies tested for hygienic behavior.

Materials and Methods:

To quantify *Varroa* and *Nosema* levels, samples of 300 adult bees were collected in alcohol jars off a comb with open larvae. To estimate the *Varroa* infestation, the alcohol samples were vigorously shaken for about one minute, the bees were thoroughly rinsed, the mites strained using a wire basket, and then counted. For six samples from each apiary, the bees were counted to get an average weight per bee. For the rest of the samples from that apiary, the bees were weighed and the weight converted to a number of bees. Dividing the number of mites by the number of bees and multiplying by 100 gave the percent *Varroa* infestation on adult bees. To correct for the mites on the pupae, the adult bee infestation was multiplied by 2 (Lee et al., 2010).

Nosema levels were estimated by reserving 100 of the 300 sampled bees. These bees were put into a plastic bag, thoroughly mashed, and 100 ml of water was added. The water and bees were mixed into slurry, and a pipette was used to collect a few drops. The drops were put on a hemocytomter slide with 25 grid squares (normally used for counting blood cells) for counting under a 400-power microscope. We counted the number of spores were counted on five of the grid squares and took an average, then a conversion factor was used to estimate the number of *Nosema* spores per bee (Cantwell, 1970). We assumed the spores found were *N. ceranae* and not *N. apis*, since *N. ceranae* is much more prevalent in the US (Klee, 2007), and is more virulent (Higes, 2007).

To test for Hygienic behavior, a comb with capped pupae was removed from each colony, a 3” PVC tube was inserted into the comb over the pupae, then 10 oz of liquid nitrogen was poured into the tube to freeze-kill the pupae. Before the addition of nitrogen, the number of cells that were not sealed pupae was counted (there are 160 cells in a 3” circle). After the tube thawed, it was removed and the comb placed back into the colony. The comb was checked in 24 hours and the number of cells not completely cleaned by the bees and the number of cells partially cleaned were counted. Since the number of initial and final cells is known, each colony can be ranked for hygienic behavior. The more cells uncapped and pupae removed, the more hygienic the colony. A hygienic breeder would completely clean out 90% or more of the sealed pupal cells.

Results and Discussion:

After the sample analysis, data from each beekeeping operation were entered into a spreadsheet and provided to the beekeepers in a timely manner – normally within a week. A rapid return of data is essential for making educated treatment decisions and choosing breeder colonies.

Each beekeeper was also given a copy of **Figures 1, 2** and **3**, which anonymously showed all the beekeeping operations *Varroa* and *Nosema* levels and the percentage of colonies tested that could be considered hygienic breeder colonies. Each beekeeper was given their own code for the figures, allowing them to see how their levels compared to the other queen producers. Disease levels and hygienic behavior levels varied greatly among the different operations. For breeding purposes, it is much more informative to have varying levels of disease in the apiary, since it reveals colonies that have high levels next to colonies with low levels. Colonies with low disease levels may have resistance traits and could be chosen as breeders, if they fit other breeder colony criteria. For hygienic behavior, two of the queen producer operations scored much higher than the rest (**Figure 3**). We will be in discussion with the beekeepers about using the scores to advertise for certified hygienic stock. In the future, we would like to connect the levels of disease with management practices, and, if given permission, share among bee breeders the management practices that are most effective and use the fewest chemical treatments.

To help interested bee breeders with genetic diversity, Lee aided S. Cobey and S. Sheppard in their project to incorporated genetic lines of New World Carniolans and Caucasians into already established bee breeder stock by helping communicate with interested bee breeders, collecting drones, and checking up on the instrumentally inseminated queens. Inseminated breeder colonies from the previous year were evaluated for general fitness to see if the colonies would be adequate to raise daughter queens.

The Bee Team will also collaborate with Dennis vanEnglesdorp (Penn State) for the implementation of the Agriculture and Food Research Initiative (AFRI) grant funded Bee Informed Partnership (BIP) (beeinformrd.org). The Bee Team was written into the grant as a way to work hands-on with the CA Bee Breeders. The Bee Team now has two new members as funded by BIP, Michael Andree and Rob Snyder.

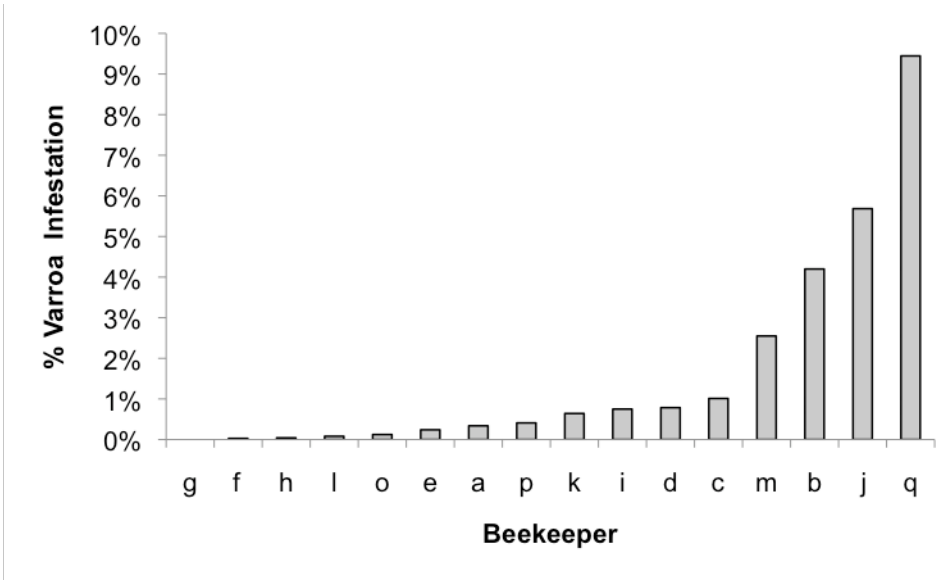

Figure 1. *Varroa destructor* infestation of adult bees (y-axis) in 16 queen producer operations, where each beekeeper is given a code (x-axis) to preserve their anonymity. Beekeepers j and q had very high levels of *Varroa*, but g and f had just treated. In the future, we will work with bee breeders to do the mite diagnostics before the colonies are treated, not after, to highlight possible resitance traits of some colonies.

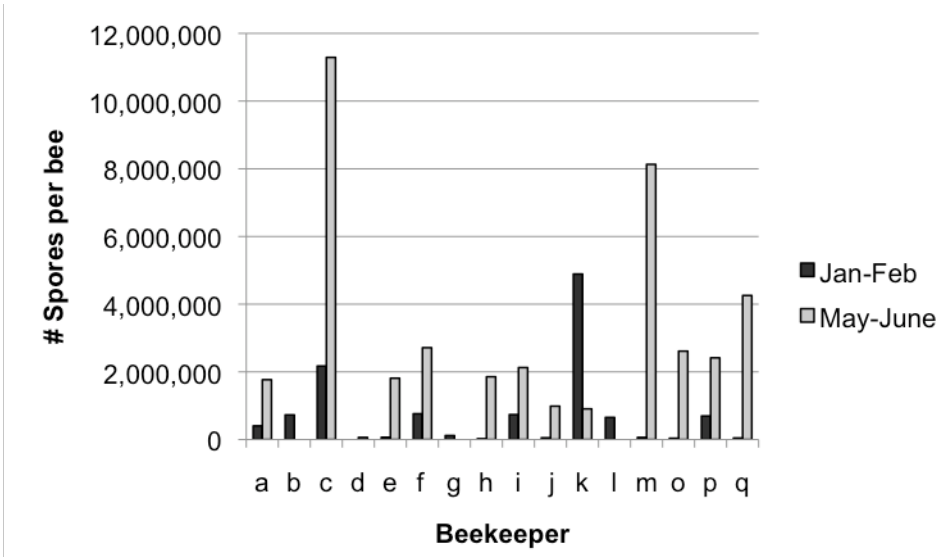

Figure 2. *Nosema* spore loads (y-axis) in 16 queen producer operations (x-axis) in the months of Jan-Feb and May-June. Each beekeeping operation is coded for anonymity. No samples were taken in Jan-Feb for beekeeper d, and no samples were taken in May-June for beekeepers b, g, and l. *Nosema* levels were higher in May-June than in Jan-Feb, except beekeeper k who had just treated repeatedly.

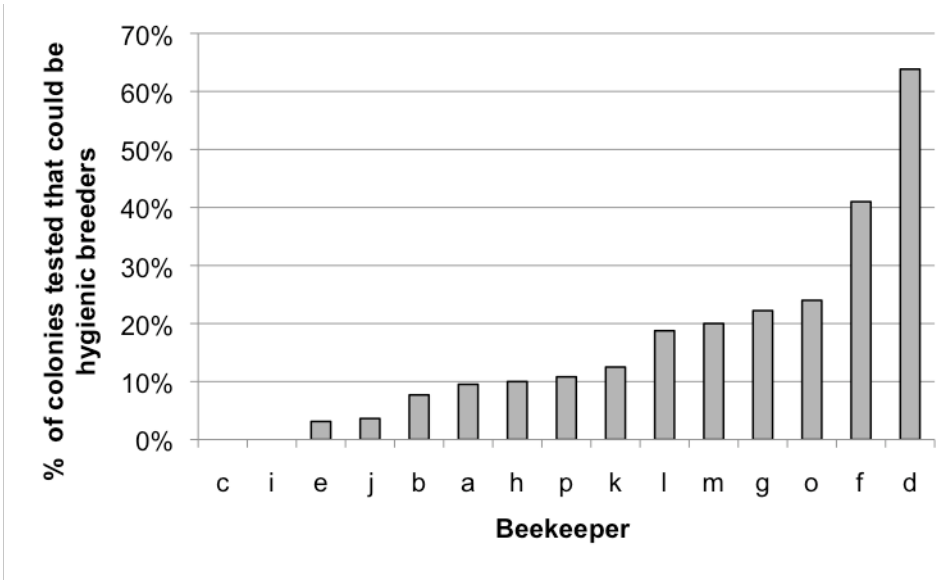

Figure 3. The percentage of colonies tested that could be considered a hygienic breeder colony (y-axis) for each of the 16 queen producing operations (x-axis). Operations f and d had over 30% of their colonies that could be certified as hygienic breeder colonies. This level of hygienic behavior is comparable to that of queen producers that have been using Spivak's inseminated breeder stock called MN Hygienics for over 10 years (Spivak et al., 2009).

Research Effort Recent Publications:

McNeil, M.E.A. 2011. The Bee Informed Partnership: a vast collaborative effort to find out what's up with the bees and your bees in particular. American Bee Journal 151(7): 677-681.

Spivak, M. 2011. Laying groundwork for a sustainable market of genetically-improved queens: The bee team. Managed pollinator CAP Update.American Bee Journal 151(5): 483-485.

Lee, K. Bee Informed Partnership: beeinformed.org/blog/. July 28, 2011. Titles: Selection for the Future is Underway, Katie Reports from the Field, Catching Queens, Drone Comets, Nosema, Cigar Smoke, Honey Bee Instrumental Insemination with Sue Cobey, Weather in Northern California, Origins, Present, Future, Rice Country, and Hygienic Behavior.

References Cited:

Cantwell, G.E. 1970. Standard methods for counting Nosema spores. American Bee Journal 110: 222–223.

Higes, M., P. García-Palencia, R. Martín-Hernándes, and A. Meana. 2007. Experimental infection of *Apis mellifera* honey bees with *Nosema ceranae* (Microsporidia). Journal of Invertebrate Pathology 94: 211-217.

Klee, J., et al. (2007) Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. Journal of Invertebrate Pathology. doi:10.1016

Lee K.V., R.D. Moon R.D., E.C. Burkness, W.D. Hutchison, and M. Spivak. 2010. Practical sampling plans for *Varroa* destructor (Acari: *Varroidae*) in *Apis mellifera* (Hymenoptera: *Apiae)* colonies and apiaries. Journal of Economic Entomology 103(4): 1039-105.

Spivak, M., and G.S. Reuter. 1998. Performance of hygienic honey bee colonies in a commercial apiary. Apidologie. 29: 291-302.

Spivak, M., G. Reuter, K. Lee, and B. Ranum. 2009. The future of the MN Hygienic stock of bees is in good hands! American Bee Journal 149(10): 965-967.