Honey Bee Stock Improvement Program

Project No.: **08-POLL4-Cobey**

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Objectives:

1. To enhance domestic honey bee stocks with an increased level of resistance to pests and diseases through selection programs and importation of honey bee semen from stock improvement programs abroad.
2. To develop commercially viable reproductive technologies and protocol for the safe importation of honey bee germplasm.
3. To provide training in techniques to advance the development and maintenance of productive commercial breeding stocks with demonstrated resistant to pests and diseases.

Interpretive Summary:

Pollination is dependent upon a healthy beekeeping industry. The impact of parasitic mites, disease and the phenomena of colony collapse disorder have and continue to greatly reduce colony numbers and weaken their effectiveness as pollinators. Honey bee diseases and parasites have largely developed resistance to the arsenal of control treatments used in colonies. Stock improvement can provide an alternative, long term and sustainable solution. Our focus is to identify, select, and enhance honey bee stocks that show increasing levels of resistant to pests and diseases. A critical aspect of this includes providing beekeeper training in stock maintenance techniques.

Genetic diversity is the basic tool for stock selection programs. Recent studies clearly demonstrate that genetic diversity is critical to colony fitness and the ability of honeybees to resist pests and disease (Sherman et al, 1988; Fuchs & Schade, 1994; Palmer & Oldroyd, 2000; Tarpy & Page, 2002; Jones et al., 2004; Tarpy & Seeley, 2006; Seeley & Tarpy. 2007; Richard, et al, 2007; Mattila et al., 2007). The decline in

genetic diversity of US honey bee populations over the past decade, as measured by genetic markers, has been demonstrated (Schiff & Sheppard, 1995 and 1996; Delaney et al., 2007). Losses are not only due to pests and diseases; the industry's use of limited breeding stock for commercial production to re-stock US colonies contributes to this "bottleneck".

The importation of stocks from bee breeding programs abroad will augment our domestic gene pool and provide access to programs selecting for increased levels of resistance to pests and disease. Standardized international protocols need to be established for the safe movement of bee stocks, as are for our nation's domestic livestock population. Reproductive technologies that decrease the risk of pathogen transmission need to be developed.

Enhancement of Commercial Stocks

To identify and encourage selection of commercial stocks with traits of resistance to pests and disease, we are working with cooperating California queen producers in collaboration with Dr. Marla Spivak. An initial survey conducted in spring of 2008 tested for hygienic behavior (a mechanism of resistance to Varroa and brood diseases) and the prevalence of Nosema and tracheal mites. Results were made available to participating producers to assist in their selection efforts. Preliminary results suggest that greater focus needs to be placed on selection for resistance to pests and disease.

Our stock importation requests to USDA-APHIS (Animal Plant Health Inspection Service) were limited to honey bee semen, in consideration of the impact of introduced pests and disease and the recognized need to minimize risks. Permits were obtained from USDA-APHIS to import semen for a three year period, 2008 - 2010, from 3 honey bee subspecies, *Apis mellifera ligustica* from Italy, *A. m. carnica* from Germany and *A. m. caucasica* from Turkey.

Honey bee viruses are known to occur in semen; therefore quarantine areas were established to isolate the imported stocks. Smoot Hill, an ecological study reserve of Washington State University, WSU, was established as the quarantine area in cooperation with, and under the supervision of Dr. Steve Sheppard.

Honey bee semen of *A.m. ligustica* was collected from survivor stock in Bari, Italy and shipped to WSU. Collaborating queen producers supplied virgin queens from their commercial stocks, which were inseminated with the imported semen. These queens were established in colonies in a WSU quarantine yard.

Honey bee semen of *A.m. carnica* from the German Carnica Association was imported from the Kirchhain Honey Bee Institute in Germany. The semen was inseminated to virgin queens from the New World Carniolan Breeding Program, NWC, maintained at UCD. These queens were established in colonies in a WSU quarantine yard.

We also plan to import semen from *A.m. caucasica* in 2009. Traces of this particular subspecies are detectable in the US gene pool, but have been largely lost. I traveled to

Turkey in August (sponsored by the TEMA foundation (http://www.tema.org.tr/) and observed several ecotypes of this subspecies. Valuable contacts and future plans are being developed.

Samples of the semen importations from Italy and Germany were sent to the USDA Beltsville Honey Bee Lab for virus testing. The Bari Italian stock tested positive for viruses and remains in quarantine at WSU. The German Carnica stock was approved by APHIS for release. This stock is currently being evaluated and propagated at UCD and will be distributed to interested California queen producers.

Reproductive Technology Development

In collaboration with reproductive specialists John Pollard, DVM and Dr. Claire Plante, DVM, we are working to develop safe and well-regulated importation protocols for bee semen and eggs. Methods for direct pathogen testing of donor colonies and gamete samples would allow for the immediate use of queens produced from imported eggs and/or semen.

For storage and shipment of bee semen, various diluents specifically designed to protect against honey bee bacterial pathogens are being tested to determine if sperm is negatively affected by various mixtures and concentrations of antibiotics.

To manage the safe movement of honey bee eggs we are developing a more practical and successful method of transfer which allow for the isolation and in-vitro hatching of eggs, their subsequent grafting, and production of viable queens. We are improving the technologies and success rate of isolating and manipulation early stage eggs. Our preliminary results demonstrate that eggs, 12-50 hours post ovipostion, can be physically manipulated without significant loss of viability. A mean hatch rate of 93% in-vitro was obtained.

Within two hours of hatching in the incubator, larvae were grafted into queen cells and introduced to queen rearing, cell builder colonies. A mean acceptance rate of 43% was obtained for in-vitro hatched larvae, which did not differ significantly from control larvae.

Queens reared from in-vitro hatched larvae appeared morphologically normal and where subsequently instrumentally inseminated. These queens were established in colonies and functioned normally in terms of initiation of egg laying and brood production. We will advance these procedures to improve the acceptance rate of in-vitro hatched larvae by cell builder colonies and their overall efficacy in the production of viable breeder queens.

Honey bee gametes are known to carry viruses. To determine the level of health risk in transporting semen and eggs we are looking at the virus profile of various aspects of colonies, using molecular techniques, such as reverse Transcription - Polymerase Chain Reaction (RT-PCR). In collaboration with Dr. Chen at the USDA Lab. and Dr. Michelle Flenniken at UCSF, we plan to determine a screening process for the presence of specific pathogenic bee viruses and investigate pathogen elimination procedures for bee gametes.

Technology Transfer

I designed three specialized beekeeping short courses to provide beekeepers with the skills required to develop and maintain honey bee stocks. Three classes were conducted at UCD, Laidlaw Honey Bee Biology Facility during the spring season, 2008. Courses are structured to provide "hands on" field and classroom training. The working model NWC breeding program is used for demonstration purposes. Beekeepers from across the country and abroad participated.

The three courses offered are:
1. The "Art of Queen Rearing" which includes a tour of several commercial queen producers in northern California
2. Instrument Insemination and Bee Breeding
3. Advanced Techniques in Instrument Insemination

These courses will be offered annually. As new technologies are developed we will incorporate these into the curriculum with the goal to enhance utilization within the beekeeping industry.

The completed report for this current 2008 – 2009 project will be available on CD and distributed at the 2009 Conference.

References:

Delaney D., Schiff NM., and Sheppard WS.(2007). Unpublished data

Fuchs, S., & Schade, V. (1994). Lower performance in honey bee colonies of uniform paternity. *Apidologie, 24*, 155-168.

Jones, C.J., Myerscough, M., Graham, S., Oldroyd, B.P. (2004). Honey bee nest thermoregulation; Diversity Promotes Stability. *Science* 305, 402-404.

Mattila, H. R., & Seeley, T. D. (2007). Genetic Diversity in Honey Bee Colonies Enhances Productivity and Fitness. *Science,* 317, 362-364.

Schiff, NM., & Sheppard, W. S. (1995). Genetic Analysis of Commercial Honey Bees from the Southeastern United States. *J.Econ. Entomol., 88*(5), 1216-1220.

Schiff, NM., & Sheppard, W. S. (1996). Genetic differentiation in the queen breeding population of the Western United States. *Apidologie, 27*, 77-86.

Sheppard, WS. 1989. A history of the introduction of honey bee races into the United States, I and II. Amer. Bee J. 129: 617-619, 664-667.

Sherman, PW., Seeley, T. D., & Reeve, H. K. (1988). Parasites, pathogens and polyandry in social hymenoptera. *Am. Nat., 131*, 602-610.

Tarpy DR, Seeley TD (2006) Lower disease infections in honeybee (*Apis mellifera*) colonies headed by polyandrous vs monandrous queens. 93: 195-199.

Seeley TD & Tarpy DR, (2007) Queen promiscuity lowers disease within honey bee colonies. Proc. Royal Society of London,B., 274:67-72.