Honey Bee Stock Improvement Program

Project No: **10-POLL4-Cobey**

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Objectives:

1. Continued development and testing of technologies for the international exchange of honey bee germplasm.
2. Enhance domestic, commercial honey bee stocks through the importation of European bee stocks from Europe.
3. Explore relationships between breeder queen and embryo pathogen status.
4. Technology transfer and stock evaluation.

Interpretive Summary:

Our approach to the current challenges facing the beekeeping industry is to enhance genetic diversity of domestic honey bee stocks through the importation of honey bee germplasm from the original homeland of the species and to assist the beekeeping industry in selection and maintenance of productive stocks with increasing resistance to pests and disease.

European honey bees were introduced into North America before the Honey Bee Act of 1922 restricted further importation. These early importations sampled a small subset of several subspecies (Sheppard, 1989). The limited foundation stock has been

propagated and expanded to establish the existing U.S. beekeeping industry. Several genetic “bottleneck” events over time have further reduced genetic diversity in the U.S. honey bee populations. The diversity of breeding stocks used by the queen and package producers, responsible for restocking colonies nationwide, is limited and decreasing (Schiff and Sheppard 1995, 1996; Delaney et al 2009). The impact of the parasitic mite *Varroa destructor* and the phenomena of Colony Collapse Disorder continue to be contributing factors to high colony losses, averaging 30% annually (vanEngelsdorp et al., 2009).

A goal of our on-going project is to develop and test protocols for the safe international exchange of honey bee germplasm, as is routine for economically valued livestock. We have successfully demonstrated that the collection and transport of honey bee semen is an effective method for exchange of honey bee stocks with minimal risk. Under USDA-APHIS permit, we have imported semen from three subspecies of European honey bees, *Apis mellifera ligustica* from Italy, *Apis mellifera carnica* from Germany and Solvenia, and *A.m. caucasica* from the Republic of Georgia.

An essential component of our project is to integrate the stocks imported from Europe into the U.S. honey bee gene pool. A collaborative program with the California Bee Breeders Association, CBBA, has been established to facilitate this effort. The distribution and evaluation of the various crosses of domestic and imported stocks is in progress to determine their productivity and acceptability by the beekeeping industry. These stocks are also being evaluated and selected for reduced prevalence of pests and diseases in a partnering effort with the Technical Transfer Team, coordinated by Marla Spivak and Katie Lee.

To provide the skills for propagation, selection and maintenance of breeding stocks we continue to offer specialty short courses annually at UCD. Four spring classes were conducted at UCD in queen rearing and instrumental insemination. Due to high demand, classes were expanded to include two additional queen rearing courses in Washington State.

This year, our project has been expanded to the Pacific Northwest, where numerous colonies are sourced for California almond pollination. We are working closely with a group of Washington beekeepers to establish local queen production. This offers testing under different climatic conditions and may result in increased availability of quality queens adapted to the PNW climate.

Materials and Methods:

1. Continued development and testing of technologies for the international exchange of honey bee germplasm.

The objective, to further develop embryo transfer technologies and develop room temperature preservation of bee semen, was initiated by John Pollard and Claire Plante, visiting scientists. They were not able to focus on this research at UCD this season.

Collaborators Steve Sheppard and Brandon Hopkins have made recent advances in the long term storage of honey bee semen. They demonstrated that successive generations of queens can be produced from semen cryopreserved in liquid nitrogen (Hopkins & Sheppard, in press). To further test various techniques and methodologies for long term semen storage, S. Cobey provided assistance in semen collection, inseminations of queens, and the establishment of colonies headed by these queens.

Honey bee semen was collected under varying conditions, stored at varying temperatures, and time intervals. To adequately test sperm viability after storage, queens were inseminated and established in colonies for observation. Colonies headed by these queens are being evaluated. Experimental queens were established at UCD, taking advantage of the early spring season. Late season queens are currently being established at WSU, testing various methodologies and time intervals of long term semen storage.

Development of improved techniques for long term storage of honey bee semen would provide a means to produce several generations a year to re-establish more pure stocks in a timely manner. The capability of long term storage would also provide a convenient and economical method of conservation of honey bee germplasm.

2. Enhance domestic, commercial honey bee stocks through the importation of European bee stocks from Europe.

In our on-going collaborative project to enhance genetic diversity in U.S. honey bee populations, honey bee semen was imported from three subspecies of European honey bees under a three year, 2008-2010, USDA-APHIS (Animal Plant Health Inspection Service) permit. Successful importations were made of *Apis mellifera ligustica* from Italy in 2008, 2009, 2010 and *Apis. M. carnica* from Germany in 2008 and 2009 and *A. m. caucasica* from the Republic of Georgia in 2010.

The USDA-APHIS semen import permit, held by W. Sheppard, was renewed for an additional three years, 2011-2013. We have sourced and established good relations with contacts in Europe to facilitate the collection of indigenous honey bee stocks. In July 2011, S. Cobey, W. Sheppard and B. Hopkins traveled to Europe to collect germplasm of two subspecies. Honey bee semen of *Apis. m. carnica* was collected from three regions in Solvenia. Semen of *A. m. caucasica* was also collected from three regions in the Republic of Georgia.

Honey bee semen collected from the various sites was stored fresh in capillary tubes at room temperature and inseminated to virgin queens reared in CA. and WA., upon return to the U.S. Portions of the collected semen were cyro-preserved in liquid nitrogen. While the methodology is experimental, this is the first time honey bee germplasm has been cryo-preserved and transported internationally.

The fresh imported semen was instrumentally inseminated to Carniolan and Caucasian virgin queens reared from progeny of the previous European imports to establish more pure stocks. Queens inseminated with the fresh imported semen are currently being established in the USDA-APHIS approved quarantine station at Bald Butte, WA.

Samples of the imported semen have been sent to Judy Chen, USDA Beltsville, for screening of viruses. The colonies established from the imported semen will be closely observed in the quarantine area for evidence of diseases. Upon release from quarantine, the stocks will be moved to locations in WA. and CA. for performance testing and propagation.

To further develop the imported Italian stock, we have incorporated the Varroa Sensitive Hygiene trait, VSH, into several lines to increase the level of resistance to pests and diseases. The USDA Baton Rouge Honey Bee Laboratory supplied fresh semen carrying the VHS trait. Bees selected for the VSH trait detect and remove a high percentage of *Varroa* infested and disease infected brood.

The *Apis mellifera ligustica* stock previously imported from Italy was propagated and virgin daughter queens were inseminated with VSH semen. The VHS semen was also mixed with fresh Park Italian bee semen and inseminated to virgins of Parks domestic Italian breeding stock to increase the level of resistance to pests and diseases. We plan to back-crossed these lines to future importations of Italian stock.

3. Explore relationships between breeder queen and embryo pathogen status.

Our currently held USDA-APHIS stock importation permit is limited to honey bee semen. For this reason, we are focusing on developing techniques for the preservation and long term storage of bee semen. Consequently, we did not further develop this objective.
Our previously reported survey of virus transmission between queens and their eggs from commercial queen rearing apiaries in northern California and Louisiana indicated the percentage of detectable virus was lower in the eggs as compared to the queens. Results suggest the mode of transmission of virus from the queen to her eggs does not appear to be strong and may vary among viruses.

4. Technology transfer and stock evaluation.

<u>Stock Evaluation & Maintenance Program</u>
To facilitate the incorporation, maintenance and distribution of the imported stocks into domestic breeding populations, a collaborative program with California Bee Breeders Association, CBBA, has been established.

Cooperating queen producers are assisting in selection and stock maintenance. Instrumentally inseminated breeder queens, established from various crosses of the imported and domestic breeding stocks, were propagated at UCD and distributed to cooperating queen producers. This year S. Cobey also conducted an insemination program for CBBA to establish various lines, which are combinations of the imported and domestics stocks, for producers to evaluate. Instrumental insemination of the Carniolan stocks were preformed at Koehnens & Sons, Inc in Ord Bend, CA. and insemination of the Italian stocks were preformed at Park's Apiaries in Palo Cedro, CA.

The various crosses of imported and domestic stocks have been established and are being

evaluated for general performance. Working closely with industry producers we are developing and implementing practical field selection criteria. To assist beekeepers in the selection process, we are partnering with the Technical Transfer Team to identify colonies expressing hygienic behavior and decreased prevalence of pests and diseases.

In preparation for future stock maintenance, CBBA queen producers will rear open mated daughter queens of the select breeders to provide drone sources for next year's insemination program. Several drone mother apiaries for each subspecies imported will be established on an annual basis.

In addition, a proposed multi-tiered program is being designed to allow for various levels of involvement for queen producers, honey producers, pollinators and sideliners.

Specialized Beekeeping Short Courses

Annually, we continue to offer and have increased the number of specialized beekeeping short courses designed to enhance the skills required to develop and maintain honey bee stocks. The annual UCD queen rearing short course includes a tour of several commercial queen producers in northern CA. The tour enables participants to observe different and successful practices involved in queen production in a short time period. Due to overwhelming interest in queen rearing, two courses were also offered in Washington State.

Six courses were conducted in 2011

1. Art Of Queen Rearing
   March 23 & 24, UCD
   March 30 and 31, UCD
   June 4, Brush Prairie, WA.
   June 15, Mt Vernon WSU Research Station.
2. Instrument Insemination & Bee Breeding Short course.
   April 13 -15, UCD
3. Advanced Techniques in Instrument Insemination
   April 20 - 21, UCD

Results and Discussion:

Historically, the beekeeping industry has not had access to long-range breeding programs. Such programs are routine for economically important, non-native livestock, including poultry, dairy, and swine. These programs are dependent upon the routine importation of genetic material from their native homelands. Genetic diversity is essential for stock improvement. The limited and declining genetic diversity in U.S. honey bee populations is a concern, as this may limit our ability to advance bee breeding efforts.

The lack of standard protocols for the safe international exchange of honey bee stocks is a limiting factor. A USDA-APHIS permit to import honey bee semen was obtained allowing the testing of a protocol for importation. Honey bee semen can be held at room

temperature for several weeks with good viability (Collins, 2003). We have demonstrated that queens instrumentally inseminated to semen, stored at room temperature for up to two weeks during transport, can successfully head productive colonies for an average of one year and some lasting over two years.

Methodology for the long term storage of honey bee semen is being developed. This would provide the ability to backcross several generations a year to re-establish more pure stocks in a single season and may establish an economical method of conservation of commercial stocks as well as indigenous ecotypes of honey bees currently threatened.

Our on-going collaborative project is designed to strengthen domestic honey bee breeding stocks through enhancement of genetic diversity and providing access to bee breeding programs abroad that are selecting for resistance to pests and disease. We have imported honey bee semen from three subspecies of endemic Old World European honey bee populations; *A.m. ligustica* from southern Italy in 2008 and 2009, and from northern Italy in 2010, *Apis mellifera carnica* from Germany in 2008 and 2009 and Slovenia in 2011, and *Apis mellifera caucasica* from the Republic of Georgia in 2010 and 2011.

Italian and Carniolan (*carnica* subspecies) stocks are the two recognizable and common subspecies kept by U.S. beekeepers. The imported German *Apis. m. carnica* stock has been incorporated into the domestic New World Carniolan line. This is currently being propagated by commercial queen producers and available to beekeepers nationwide. The *Apis. m. carnica* stock from Slovenia will also be incorporated into this program.

This year our collection efforts have focused *Apis. m. carnica* and *A. m. caucasica.* These two subspecies are well adapted to cold, harsh climates and forage under marginal weather conditions. The characteristics of these honey bees may benefit their effectiveness as pollinators for the early spring almond bloom.

While traces of Caucasian (*caucasica* subspecies) bees are detectable in the U.S, this subspecies is not recognizable. We are re-establishing *A. m. caucasica* in the U.S. Caucasian bees are known for their collection of propolis, which contains antimicrobial properties that act against bacteria, fungi and viruses. This factor may contribute to lessening the impact of honey bee pathogens.

A critical component of this project is incorporation of the imported European stocks into domestic U.S. breeding populations and the acceptance of these stocks by the commercial queen producers. Therefore a collaborative program involving the California Bee Breeders Association has been established and is developing to make and test various crosses of the stocks and to evaluate, select, maintain and develop these lines over time. This effort is highly collaborative involving industry, UCD and WSU and the Technical Transfer Team.

Research Presentations:

Development of Protocol for the International Exchange of Honey Bee Embryos. American Assoc. of Prof. Apiculturists. Jan. 6, 2011.

WSU & UCD Cooperative Honey Bee Stock Improvement Project. American Beekeeping Federation. Jan. 4-8, 2011.

Stock Importation Update and Future Possibilities. California State Beekeepers Assoc. Nov. 16-18, 2010

Mating Is Risky Business and the Benefits of Being Promiscuous. CalPoly Horticulture & Crop Science Dept. Dow Agrosciences Seminar Series Nov. 15th, 2010.

Honey Bee Stock Improvement Project. Iowa State Beekeepers Assoc. Nov. 6, 2010

The Challenge of Stock Improvement. Northwest Corner Beekeeping Conference. Mt Hood, OR. Oct.28-30, 2010

Selection of Honey Bees for the Pacific Northwest. Whatcom, Skagit, Island County and Mt Baker's Beekeepers Assoc.. Mt Vernon, WA. . Oct.13, 2010

Honey Bee Breeding & Why Our Progress is Slow. Western Apicultural Society Aug 31, 2010

La Inseminación Instrumental en Mantener Diversidad Genética y Programas de Mejora de la Acción de las Abejas Europeas de la Miel. XXIV Seminario Americano de Apicultura . Cuernavaca, Mexico July 26-31, 2010.

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