Research Project Report

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Testing Protocols For The Safe International Exchange Of Honey Bee Stocks To Enhance Proven Commercial Lines Through Selection and Increased Genetic Diversity

Final Report
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Statement of Problem And Approach

The complex phenomena of Colony Collapse Disorder, CCD, continues to weaken colonies and the ability of the beekeeping industry to supply the demand for pollination services required by production agricultural for vegetable, fruit and seed crops (VanEnglesdorp, et.al., 2009 and 2010).

A concern in the continuing high loss of colony numbers, is the use of limited breeding stock by commercial queen producers responsible for re-stocking colonies nationwide (Delaney, 2009). A limited domestic honey bee gene pool may slow our ability to advance stock improvement programs. It is well documented that genetic diversity is critical to colony fitness and the ability of honey bees to resist pests and diseases (Sherman, et al, 1988; Fuchs & Schade, 1994; Palmer & Oldroyd, 2000; Tarpy & Page, 2002; Tarpy & Seeley, 2006; Seeley & Tarpy. 2007; Richard, et al, 2007; Mattila et al, 2007).

Our goal is to enhance genetic diversify of domestic honey bee breeding populations and take advantage of the progress made in selection programs abroad. To provide access to stocks of European honey bees, not native to the US, there is a need to develop technical and legal protocol for the safe international exchange of honey bee germplasm. The technology available to facilitate the safe and practical movement of honey bees is limited. The current regulatory standards are inconsistent and have not prevented the spread of honey bee pests, parasites and pathogens.

An important aspect of this project includes working closely with the industry. There is a need to assist the industry in the development, selection and maintenance of productive breeding stocks that show increasing levels of resistance to pests and diseases. The transfer of essential skills and technologies to advance the beekeeping industry in their breeding efforts is a critical component.

Objectives & Results

Our objective is to develop and test reproductive technologies for the safe international exchange of honey bee stocks and to assist the U.S. queen producers in the enhancement of their breeding programs. We are providing access to honeybee germplasm from Europe and ensuring the long term maintenance of these various stocks. Our cooperative project is supported in part by the Almond Board of California and the Foundation for the Preservation of Honey Bees.

Working closely with cooperating queen producers, we are incorporating several lines of imported stocks into established breeding populations of U.S. commercial stocks. To provide technology transfer we continue to work closely with industry and to offer short courses in the specialized skills necessary for stock selection and maintenance.

Additionally, we have joined COLOSS, www.coloss.org, a global network of honey bee researchers, beekeepers and industry members working to identify the complex of factors involved in CCD and develop sustainable management strategies to prevent colony loss. One aspect of this is to develop recommended standards for international movement of honey bee to OIE, the World Organization for Animal Health.

Stock Enhancement Program

We have successfully demonstrated that the collection and transport of honey bee germplasm is an effective method for exchange of honey bee stocks with minimal risk. Under USDA-APHIS permit, a protocol for importation of honey bee semen has been approved. Samples of imported semen are tested for viruses. An approved isolated quarantine area has been established in Washington State to initially establish, observe and test the stocks.

We have imported semen from three subspecies of European honey bees and have or are in the process of incorporating these into domestic commercial breeding programs to enhance genetic diversity in the U.S. honey bee gene pool. The long term goal includes assisting the industry in the development, selection and maintenance of these various stocks.

Apis mellifera carnica

Honey bee semen of *A.m. Carnica* was imported from Germany in 2008 and 2009 and from Slovenia in 2011. The German stocks have been incorporated into the domestic New World Carniolan Closed Population Breeding Program, NWC. Production queens resulting from these crosses have been widely distributed by commercial queen producers with positive results. Beekeepers using the enhanced NWC stock notice the change and their anecdotal reports claim increased productivity, wintering ability and reduced Varroa mite growth. Our most recent importation of Slovenian stock, in July of 2011, has just been released from quarantine and will be incorporated in the NWC in 2012.

Apis mellifera ligustica

Importation of honey bee semen of *A.m. ligustica* from Italy was imported in 2008, 2009 and 2010. Queens reared from several domestic Italian stocks were supplied by the industry and crossed. We also backcrossed stocks imported in 2010 to progeny resulting from the previous importations.

During the spring season of 2011 we incorporated the trait, Varroa Sensitive Hygiene, VSH, into the Italian lines to increase the level of resistance to pests and diseases. The *Apis mellifera ligustica* stock 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. 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 diseased brood.

Apis mellifera caucasica

Caucasian honey bees, *A.m. caucasica,* are a dark, winter hardy subspecies and avid collector of propolis. Propolis is known for its antimicrobial properties and quieting the immune system (Simone et al, 2009), pictured in Figure 1a. This subspecies is currently unrecognizable in the US, although genetic traces can be found. To re-establish *A.m. caucasica,* semen from the Republic of Georgia was imported in 2010 and 2011. Drones were collected from several regions, including an apiary in Mestia, Georgia,

pictured in Figure 1b. The initial 2010 importation was mated to Carniolan queens. Daughter queens of these crosses were reared and inseminated to the 2011 importations. Future importations are planned to further develop this stock.

Fig. 1a Frame with Propolis from an *A.m. caucasica* colony. Propolis is known for its antimicrobial properties.

Fig. 1b Collection of drones in an apiary of *A.m. caucasica* in Mestia, Republic of Georgia.

Cooperative Stock Evaluation & Maintenance Program

An essential component of our on-going program is to facilitate the incorporation, maintenance and distribution of the imported stocks into domestic breeding populations. A collaborative program with the California Bee Breeders Association, CBBA has been established. We are providing stocks, an insemination service and selection methodology to establish and maintain the various commercial lines, enhanced by the imported stocks. We are also partnering with the California Technical Transfer Team, headed by Marla Spivak, to assist in the evaluation of the various stocks. Selection for productivity and identifying colonies expressing hygienic behavior and decreased prevalence of pests and diseases is being conducted. Cooperating CBBA members will be responsible for the production and nationwide distribution of queens reared from these select stocks.

In addition, a multi-tiered program to allow for various levels of involvement for queen producers, honey producers, pollinators and sideliners is being developed and implemented. A queen rearing group has been established in Washington State to propagate the imported stocks for the Pacific Northwest. We plan to develop and expanded this aspect to encourage regional production of queens by smaller producers to address the increasing need and demand for high quality queens.

Development & Testing of Reproductive Technologies And Importation Protocols

Cyro-preservation of honey bee semen

The collection of semen is seasonally restricted to drone availability and requires specialized equipment and skill to collect. Development of techniques for Cyro-preservation of honey bee semen would provide a convenient and economical method of long term storage.

Considering our USDA importation permit is restricted to honey bee semen, we are exploring the feasibility of long term semen storage. In collaboration with Brandon Hopkins, a graduate student at Washington State University, a portion of the semen collected during our 2011 trip was stored in liquid nitrogen. Collections include *A. m. carnica* from Slovenia and *A. m. caucasica* from the Republic of Georgia. While somewhat experimental, we hope to use the stored semen for future backcrosses to increase the purity to these subspecies. Various methodologies to advance techniques for long term semen storage are being tested in collaborative efforts between WSU and UCD.

Egg Transfer Techniques

We developed a method to transfer eggs in isolation, removing them from their beeswax base. Honey bee eggs are delicate and the basal ends are glued to the comb, making removal without damage difficult. Considering honey bee eggs represent a complete genetic package, are available in large numbers and the three day stage allows time for transport, eggs are a good candidate for genetic exchange.

Our egg manipulation technique uses a pair of micro-forceps with the attachment of flexible micro-bore tubing to the distal pincers. The apical end of the egg is gently grasped between the tubing and lifted to separate the glued basal end of the egg from the brood comb, pictured in Figure 2. Using this technique eggs are easily transferred to sterile plastic plates and maintained in their upright position.

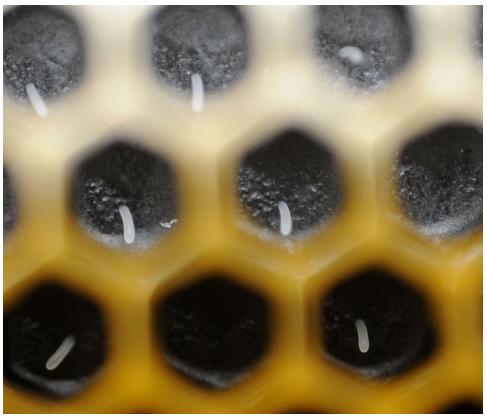
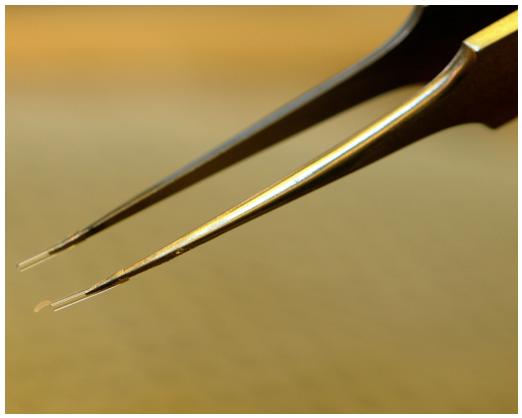


Fig.2a.Honey bee eggs laid on beeswax comb.



2b. Micro-forceps with attached flexible micro-bore tubing and egg.

2c. Transfering eggs using the modified forceps technique.

Figure 2: The egg transfer technique uses micro-forceps with attached flexible micro-bore tubing to the distal pincers.

To develop reproductive technologies and regulatory protocols to minimize the risk of pathogen transfer during importation, an understanding of the pathogen relationship between various reproductive stages of individual colonies is needed. The risk of pathogen transmission in gametes was explored to develop procedures for certification of breeder colonies intended for germplasm donation. Our preliminary sampling, using RT-PCR analysis, indicated transmission of virus varies among hive constituents within a single colony judged to be healthy and without symptoms of disease, other than Varroa.

Importation of germplasm minimizes the transmission risk of pests, parasites and pathogens, although transmission of virus remains a concern. The relationship and pathogen status of the queen, drones, eggs, semen and drone feces within individual colonies is unknown. To explore this question, we collected samples of these various constituents from colonies of several large California queen producers for viral analysis.

Samples were analyzed using the BEE CHIP, a bee pathogen microarray chip developed by Dr. J.DeRisi's Lab. at University of California, San Francisco. The BEE CHIP, originally designed for adult bee sampling, proved unreliable in processing of other hive constituents. The BEE CHIP involves a hybridization technique detection that is not only based on whether a pathogen is present, but is also depends on the relative amount of pathogen and host material isolated from each sample. The technique was not sensitive enough for some of the hive constituents. Obtaining enough RNA from samples of embryo, feces, and semen proved difficult. The high fat content of immature bee stages was also problematic in processing samples.

With use of the BEE CHIP in control experiments, that were known to be positive for at least four honey bee viruses, the viruses were no longer detectable in samples if the

starting material was reduced to 100 ng or below. For these reasons we repeated the sampling in spring 2010 and used reverse Transcription - Polymerase Chain Reaction (RT-PCR) analysis.

During March and April 2010, we collected samples from breeding populations of commercial stocks in California and Louisiana to determine the presence and transmission of viruses between queens and eggs. One hundred colonies were sampled, taking the queen and 50 of her eggs from each colony. The samples represent nine commercial breeding populations from the major queen production areas of the country.

The samples were screened for eight known viruses using RT-PCR. Analysis was conducted by Dr. Chen, at the USDA Beltsville Honey Bee Laboratory. Our results, as seen in Table 1, indicate that of the eight viruses tested, only three viruses, BQCV, DWV and IAPV, were detected in both queens and eggs. Although we do know the viral status of the colonies, five viruses; ABPV, CBPV, KBV, SBV, and SPV were not detected in either queens or eggs. This may indicate that the mode of transmission from various constituents of the colony to the queens is reduced, although further testing is needed to clarify this aspect.

The percentage of detectable virus was lower in the eggs as compared to the queens. DWV was the most prevalent virus, found in 95% of the queens and 56% of the eggs. BQCV was detected in 88% of queens and only 3 % of the eggs. To confirm these results, the assay to detect the rate of BQCV in eggs was repeated and did yield the same results. IAPV, although less prevalent by comparison to DWV and BQCV, appeared to have a higher rate of transmission, detected in 27% of queens and 23% of their eggs. Overall, these preliminary 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.

	ABPV	BQCV	CBPV	DWV	IAPV	KBV	SBV	SPV
Queens	0%	88%	0%	95%	27%	0%	0%	0%
Eggs	0%	3%	0%	56%	23%	0%	0%	0%

Table 1: Transmission of viruses between queens and their eggs determined by RT-PCR analysis. The percentage of detectable viruses found in the queen and 50 of her eggs from 100 colonies representing breeding populations in California and Louisiana.

Technology Transfer

To provide technology transfer of the skills required for honey bee breeding and stock maintenance, we continue to offer and develop specialized beekeeping short courses every spring. Our focus is to provide practical training and encourage the establishment of long term honey bee stock improvement programs, with a focus on resistance to pests and diseases. Training courses continue to be offered annually at the Laidlaw Bee Research Facility at the University of California, Davis. We are expanding to also offer these classes in Washington State.

The focus of the courses is to provide the skills required for queen rearing, applied selection and breeding methodology and the technique of instrumental insemination. Participants included beekeepers and researchers from across the country and abroad.

Courses conducted in 2011

1. Art Of Queen Rearing
   March 23rd & 24th, UCD
   March 30th and 31st, UCD
   June 4th, Brush Prairie, WA.
   June 15th, Mt Vernon WSU Research Station.
2. Instrument Insemination & Bee Breeding Short course.
   April 13th, 14 th & 15th , UCD
3. Advanced Techniques In Instrument Insemination
   April 20th & 21st., UCD

Future Plans

This project is part of a long term effort focused on honey bee stock improvement. Our goal is to encourage and support industry bee breeding programs and the enhancement of genetic diversity through the incorporation of germplasm from superior stocks imported from Europe. The development of protocols for the transport of honey bee germplasm will provide access to international genetic improvement programs.

The success of this program depends upon acceptance by the beekeeping industry, therefore we are working closely with queen producers to evaluate and maintain the imported stocks and incorporate these into established commercial breeding programs. Annually, we will continue to offer the specialized beekeeping classes designed provide skills in bee breeding and stock maintenance.

Presentations

American Assoc. of Prof. Apiculturists. Jan. 2011
American Beekeeping Federation. Jan. 2011.
California State Beekeepers Assoc. Nov. 2010
CalPoly Horticulture & Crop Science Dept. Dow Agrosciences Seminar Series Nov. 2010.
Iowa State Beekeepers Assoc. Nov. 2010
Northwest Corner Beekeeping Conference. OR. Oct., 2010
Whatcom, Skagit, Island County & Mt Baker's Beekeepers Assoc.. WA. . Oct. 2010
Western Apicultural Society Aug, 2010
XXIV Seminario Americano de Apicultura . Cuernavaca, Mexico July, 2010
Buckfast Bee Breeders Assoc. Aua, Germany Mar., 2010
American Assoc. of Professional Apiculturists. Jan. 2010
American Beekeeping Federation Jan. 2010
American Honey Producers Assoc. Jan. 2010
California Almond Board Conference - Dec. 2009
California State Beekeepers Assoc. Nov. 2009
California Bee Breeders Assoc. Oct. 2009
Apimondia International Beekeeping Congress, Montpellier, France, Sept. 2009

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