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# Honey Bee Stock Improvement Program

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**Project No:** 11-POLL4-Cobey

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**Collaborating Personnel:**

Steve Sheppard and Brandon Hopkins, Washington State University  
Elizabeth Frost, University of California, Davis  
Ca. Tech Transfer Team and BIP, Bee Informed Partnership  
supervised by Marla Spivak of Univ. of MN.  
and Dennis vanEngelsdorp, Univ. of MD.  
Dr. Judy Chen, USDA, Beltsville, Md.

**OBJECTIVES:**

1. Continue the importation of germplasm from endemic populations of European honey bees to enhance domestic honey bee breeding stocks and provide access to programs abroad selecting for resistance to pests and diseases.
2. Continue to develop and implement cooperative Industry and University based programs to propagate evaluation and maintain commercial honey bee breeding stocks and incorporate the imported stocks into these programs.
3. Advance Technologies for the International Exchange of Honey Bee Germplasm.
4. Continue for provide and expand the specialized Tech. Transfer short courses.

**Interpretive Summary:**

The initial and limited introduction of honey bees into North America and several genetic “bottleneck” events over time have reduced genetic diversity in U.S. honey bee populations. Of concern is the ability to select honey bees that are hardy, productive and show an increased level of resistance to pests and diseases from within this reduced gene pool. Our goal is to enhance the diversity of domestic honey bee breeding stocks used by the queen and package bee industry, responsible for restocking colonies nationwide. Genetic diversity, the raw tools for selection, has clearly been show to increase colony fitness and lessen the severity of pests and diseases. To accomplish this we have established a cooperative University and industry program, working closely with beekeepers.

Our on-going project provides access to endemic Old World European honey bee

populations and breeding programs that are selecting for resistance to pests and disease. The germplasm of three sub-species of European honey bees has been successfully imported; *Apis mellifera ligustica* from Italy, *Apis mellifera carnica* from Germany and Slovenia, and *A.m. caucasica* from the Republic of Georgia. This year we have concentrated on the collection of *Apis mellifera ligustica* from Italy.

To ensure the success of this program we are working closely with the California queen producers. The imported stocks have been incorporated into domestic commercial bee breeding programs in California. In a cooperative effort we are also assisting in the selection and maintenance of these breeding stocks with a focus on increasing resistance to pests and diseases.

To enhance beekeeper skills required for the propagation, selection and maintenance of breeding stocks specialty short courses are conducted annually in California and Washington. These classes are popular with waiting lists for next year.

### **Materials and Methods:**

#### **1. Importation of Germplasm**

Honey bee germplasm, specifically bee semen, has been sourced and imported from three subspecies of endemic Old World European honey bee populations in a collaborative UCD and WSU effort (Please see the complimentary WSU Report for more details). The USDA-APHIS import permit, held by Steve Sheppard at WSU, had been renewed through 2013. This year we made a collection trip to Italy to obtain *Apis mellifera ligustica* germplasm.

#### ***Apis mellifera ligustica***

Honey bee semen of *A.m. ligustica* was initially imported from Bari in southern Italy in 2008 and 2009; and from Bologna in northern Italy in 2010. Additional germplasm from several locations in Italy was collected and successfully imported in June 2012. Virgin queens supplied by cooperating California producers were inseminated with the imported semen and established in the USDA approved quarantine apiary in Washington. Some of these queens were progeny of the earlier imports from Italy, backcrossed to establish a more pure line, as well as incorporated into domestic stocks.

Samples of the imported germplasm were sent to Judy Chen at the USDA Beltsville Laboratory to test for viruses. In the quarantine area, colonies were closely observed for evidence of diseases. This stock has been released from quarantine and the colonies will be moved to locations in WA. and CA. for performance testing and propagation.

#### ***Apis mellifera carnica***

Importations of honey bee semen from *Apis mellifera carnica* was imported from Germany in 2008, 2009, and from Slovenia in 2011. These importations have been incorporated into the New World Carniolan Closed Population Breeding Program and distributed to California queen producers. An annual insemination program has been implemented with cooperating California producers to establish various crosses of the imported and domestic Carniolan stocks. The evaluation for performance testing of

these stocks is ongoing. Breeder queens from this stock have been selected and are being used for the propagation of production queens distributed to the beekeeping industry nationwide.

### ***Apis mellifera caucasica***

Importations of honey bee semen from *Apis mellifera caucasica* from the Republic Georgia was collected and imported in 2010 and 2011. During the active 2012 spring season, backcrosses have been made between these to re-establish this subspecies in the U.S. Caucasian bees were currently not recognizable in the U.S, until now. This stock is being maintained and tested in WA. and some queens have been distributed to cooperating queen producers in CA. for evaluation. In addition, crosses of the two dark races of bees, Carniolan and Caucasian, have also been established for evaluation.

## **2. Incorporation of Imported Stocks into Domestic Breeding Populations and Implementation of a Cooperative Stock Maintenance Program**

To increase the genetic base of U.S. honey bee breeding stocks, the honey bee semen importations have been inseminated to domestic honey bee queens and incorporated into domestic bee breeding programs in cooperation with cooperating California producers. Various crosses of the imported and domestic stocks have been established. An annual instrumental insemination program for the CA. queen producers has been implemented to propagate and maintain these stocks. The resultant queens have been distributed, evaluated and utilized as breeding stock for large scale propagation. The Bee Informed Partnership, BIP, and the Ca. Tech Transfer Team are assisting in the evaluation process with a focus on selection for increased tolerance/resistance to pests and diseases. To maintain and select superior stocks, cooperating California queen producers annually rear virgin queens and drones for the insemination program. Apiaries of drone mother colonies are also being established to provide drone production representing the three subspecies for the 2013 season insemination program.

To further evaluate the various imported stocks in different environments, and to provide reservoirs for re-selection purposes, we are working with honey producers and pollinators in Washington and Oregon. Regional queen rearing efforts have been promoted and established.

## **3. Advance Technologies for the International Exchange of Honey Bee Germplasm**

To ensure the imported germplasm is available for future breeding purposes, bee semen collected from the Old World European stocks was also cryopreserved in liquid nitrogen. In collaboration with WSU, recent advances made in this technique (Hopkins & Herr, 2010 and Hopkins et al 2012) were utilized and proved successful. Queens reared this season, resulting from previous imports, have been successfully backcrossed to the semen collected in 2011 and cryopreservation in liquid nitrogen. This technology provides a practical and economical method for long term storage and provides a foundation to establish a future honey bee germplasm repository.

## **4. Tech. Transfer short courses**

Specialized beekeeping short courses are offered annually in California and Washington to promote queen rearing and enhance the skills required to develop and

maintain honey bee stocks. The CA. 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 large scale queen production. The Washington State classes have resulted in several cooperative local queen rearing efforts. These also offer a means for sideliners and small beekeepers to take advantage of utilizing the imported stocks. The courses have been well received with waiting lists for the next season.

### **Courses Spring**

Queen Rearing Workshop. UCD Mar 27 & 28<sup>th</sup> 2012

Instrumental Insemination. UCD April 17, 18 & 19<sup>th</sup>, 2012

Instrumental, Advanced UCD Apr. 25<sup>th</sup> & 26 2012

Skagit Beekeepers Field Day. WSU Mt Vernon Research & Agriculture Station  
Aug. 18<sup>th</sup>, 2011

Queen Rearing Workshop. WSU, Brush Prairie, WA. June 2, 2012.

Queen Rearing Workshop. WSU Mt Vernon, WA. June 5, 2012.

Queen Rearing Workshop. Mt. Vernon, WA. , WA. June 15, 2011

Queen Rearing Workshop. Brush Prairie, WA. June 4, 2011

### **Results and Discussion:**

Three Old World European subspecies of honey bees have been successfully imported as germplasm and incorporated into domestic bee breeding programs in a cooperative University and Industry effort. The *Apis mellifera carnica* imported from Germany and Slovenia has been incorporated into the New World Carniolan Closed Population Breeding Program. Anecdotal reports by beekeepers indicate a noticed increase in productivity and survivability. The *A.m. caucasica* imports from the Republic of Georgia have resulted in re-establishing this subspecies in the US. Caucasian bees, a dark winter hardy race, produce a lot of propolis, known for its antimicrobial activity and suppression of pathogens. The Italian bee, the most popular commercial bee, has been genetically enhanced by the addition of *Apis mellifera ligustica* from Italy.

A long term program to propagate, evaluate and maintain these various stocks is being implemented. This critical aspect requires working with and gaining the confidence of the beekeeping industry to integrate the imported European stocks into the U.S. honey bee gene pool. A collaborative program with the California Bee Breeders Association has been established. The distribution and evaluation of the various crosses of domestic and imported stocks is on-going to determine their productivity and acceptability by the beekeeping industry. These stocks are also being evaluated for prevalence of pests and diseases in a partnering effort with the BIP and the Technical Transfer Team, to help beekeepers select for resistance /tolerance to pests and diseases.

We have successfully demonstrated that the collection and transport of honey bee semen is an effective and safe method for exchange of honey bee stocks. In addition,

we have shown that honey bee semen collected and cryopreserved in liquid nitrogen is a practical method for long term storage. Considering the seasonal constraints of rearing queens and drones and the limitations of accessing semen only from stocks abroad, this provides a major advance.

Specialty short courses, designed to provide the skills for propagation, selection and maintenance of breeding stocks are offered annually in CA. and WA. These have been well received and resulted in several cooperative local and regional queen rearing efforts.

## **Research Presentations:**

### **Invited Seminars – International**

Enhance of Commercial Breeding Stocks. British Columbia Beekeepers, Assoc. Vancouver, Canada. Oct. 20-23, 2011.

Honey Bee Breeding. Seminar. Universidad Mayor, Ciencias Silvoagropecuarias. Santiago Chile. Oct. 8, 2011

Developments and Changes in the Queen & Package Bee Industry. Keynote Speaker, Symposium Live Material. Apimondia International Beekeeping Congress. Buenos Aires, Sept. 19-23, 2011

### **Invited Seminars – National**

UCD Dept. of Entomology Seminar. Enhancing Genetic Diversity in the US Honey Bee Gene Pool. May 2, 2012

WSU Bee Breeding Project, Update. Skagit Valley Beekeepers, Mt Vernon, WA. Feb. 9th, 2012

Queens, Queens, Queens. Whidbey Island Beekeepers Assoc. Feb. 1, 2012.

Stock Maintenance and Distribution Program Update. California Bee Breeders Assoc. Ord Bend, CA. Jan. 18, 2012

Importation of Honey Bee Germplasm to U.S. Enhance Breeding Stocks. American Beekeeping Federation. January 12, 2012. Phoenix, AZ

The introduction of Honey Bee Germplasm and Re-establishment of *Apis mellifera caucasica* American Honey Producers Assoc. Jan. 7, 2012. Las Vegas, NV.

Queens Rearing and Introduction of Germplasm. Pierce County Beekeepers. Puyallup, WA, Jan. 2, 2012

Honey bee stock improvement. Poster. Almond Board of California. Modesto, CA. Dec. 7- 8, 2011

Enhancement of U.S. Breeding Stocks. Oregon State Beekeepers Assoc. Seaside, OR, Nov. 18-19, 2011

Queen Rearing Workshop. California State Beekeepers Assoc. Rohnert Park, CA. Nov. 15-17, 2011

Propagation and distribution of novel genetic material to beekeepers, Washington State Beekeepers Assoc. Oct. 27-29, 2011

Honey Bee Stock Improvement “Brown Bag” Seminar. WSU Mt Vernon Research &

Agriculture Station Aug. 9, 2011

### **Publications, Books and Chapters**

- Cobey, S., Sheppard, W. Tarpy, D. 2011. Status of breeding practices and genetic diversity in domestic honey bees. Chap. In: Honey Bee Colony Health: Challenges and sustainable Solutions. Ed. Sammataro, D. & Yoder, J. Pp.25-36. CRC Press
- Cobey, S., 2011. Artificial Insemination of Queen Bees. In: The Vet and the Bee. World Organization for Animal Health. OIE Technical Series no<sup>o</sup> 12. Ed. Wolfgang Ritter.

### **Literature Review**

- Delaney, D. A., M. D. Meixner, N. M. Schiff and W. S. Sheppard. 2009. Genetic characterization of commercial honey bee (Hymenoptera: Apidae) populations in the United States by using mitochondrial and microsatellite markers. *Ann. Entomol. Soc. Am.* 102: 666-673.
- Hopkins, B.K., Herr, C., Sheppard, WS. Sequential generations of honey bee (*Apis mellifera*) queens produced using cryopreserved semen. In Press.
- Hopkins, B.K. and Herr, C. 2010. Factors affecting the cryopreservation of honey bee spermatozoa. *Apidologie* 41(5) 548-556.
- Sheppard, W.S. 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.
- Schiff, N.M., Sheppard, W.S., G.M. Loper and H. Shimanuki. 1994. Genetic diversity of feral honey bee (Hymenoptera: Apidae) populations in the southern United States. *Ann. Entomol. Soc. Am.* 87:842-848.
- Schiff, N.M., & Sheppard, W. S. (1995). Genetic Analysis of Commercial Honey Bees from the Southeastern United States. *J.Econ. Entomol.*, 88(5), 1216-1220.
- Schiff, N.M., & Sheppard, W. S. (1996). Genetic differentiation in the queen breeding population of the Western United States. *Apidologie*, 27, 77-86.
- vanEngelsdorp, D., J. D. Evans, C. Saegerman, C. Mullin, E. Haubruge, B. K. Nguyen, M. Frazier, J. Frazier, D. Cox-Foster, Y. P. Chen, R. Underwood, D. R. Tarpy, and J. S. Pettis. (2009). Colony Collapse Disorder: A Descriptive Study. *PLoS ONE*, 4.