Importation and Preservation of Germplasm for US Honey Bee Breeding and Stock Improvement

Project No.: **11.POLL7.Sheppard**

Project Leader: Walter S. (Steve) Sheppard
Department of Entomology
Washington State University
Pullman, WA 99164
509.335.0481
shepp@wsu.edu

Project Cooperators and Personnel:

Sue Cobey, UC Davis and Washington State University
Brandon Hopkins, PhD candidate, Washington State University
Dr. Judy Chen, USDA-ARS, Beltsville, MD
Ca. Tech Transfer Team and Bee Informed Partnership, supervised by Marla Spivak, Univ. MN and Dennis vanEngelsdorp, Univ. MD

Objectives:

1) Continue the collection of germplasm from endemic populations of European honey bees and import into the US to enhance genetic diversity of domestic honey bee breeding stocks
2) Implement cryopreservation of all collected honey bee germplasm for both immediate and long term breeding use from a variety of Old World source populations of the three permitted honey bee subspecies.
3) Continue a selective breeding program to evaluate and improve introduced stocks and hybrids under US conditions, screening especially for resistance to pests and diseases
4) Develop and implement a cooperative Industry/University based program to disseminate appropriate stocks from the imported honey bee germplasm and assist in the evaluation and maintenance of desirable breeding stocks.

Interpretive Summary:

This document represents the final progress report for an award of the Almond Board to W.S. Sheppard to assist with expenses necessary to import and disseminate novel honey bee germplasm. Honey bee germplasm (semen) from Old World original source populations in Italy was collected in June of 2012 and hand-carried under USDA-APHIS permit into the United States. The semen was used to instrumentally inseminate domestic virgin queens produced and supplied by collaborating California queen breeders. The inseminated queens will be maintained over the coming winter (2012-2013) in WA, ID and CA and will be made available to commercial queen producers in Spring 2013 to enhance the diversity of US honey bee populations. Previous germplasm (collected and used for insemination in 2011) was maintained in colonies in a similar manner and distributed in 2012. This project provides a mechanism for ongoing and future introductions of honey bee germplasm for the genetic improvement of commercial honey bee populations, a critical resource used to pollinate almonds and many other agricultural crops.

Maintaining adequate genetic diversity is fundamental to breeding programs directed toward the improvement of all crops and animals of agricultural significance. More than 30 years ago, resident strains of almonds were evaluated in 10 Mediterranean and Asian countries and this genetic source material was available by exchange to UC-Davis plant breeders (Kester and Asay, 1977). In fact, given that many crops have non-U.S. origins, the U.S. National Plant Germplasm System maintains over 500,000 accessions (samples) of seed, tissues and plants for plant breeders to use and still conducts an average of 15 expeditions per year to foreign countries to gather new genetic material (O'Brien, 2010). Similarly, breeding programs of economically important, livestock species, such as poultry, dairy, and swine rely on the importation of genetic material from within the original ranges of the species. Historically, the beekeeping industry has not had access to these sorts of genetic resources, a limitation that could limit the ability off bee breeders to select for resistance to *Varroa* and other pests and diseases.

Materials and Methods:

In 2012, WSS, Sue Cobey (SWC) and Brandon Hopkins (BKH) traveled to Italy and made a number of collections of semen from the subspecies *Apis mellifera ligustica* from 6 distinct locations ranging from Ariano-Irpino in the south to Reggio-Emilia (Bologna) in the north. Of especial interest were two collecting locations made from apiaries maintained by the Italian federal laboratory on honey bee genetic diversity. The genetic stocks sampled in the federal bee laboratory sites included a collection of authenticated sources of the subspecies *A. m. ligustica*, the strain of honey bee predominantly favored by US beekeepers. Collected germplasm (semen) was returned to the US in late June 2012 under a USDA-APHIS hand carry permit awarded to WSS. California Queen producer collaborators had pre-shipped virgin honey bee queens of US "domestic Italian" stocks to WSU and over 60 of these queens were inseminated with the imported Italian honey bee semen. SWC conducted the instrumental inseminations and served as the primary industry liaison in the project to acquire the virgin queens. Aliquots of semen were concurrently supplied to Dr. Judy Chen of the USDA-ARS Bee Research Laboratory in Beltsville MD for virus determination. Collection and introduction of genetic material derived from Old World endemic honey bee populations into the US has been ongoing under this project since 2008. To date, we have imported semen from three subspecies of high importance to the US beekeeping industry, including *A. m. ligustica* (Italian), *A. m. carnica* (originally derived from the Alps) and *A. m. caucasica.*

Results and Discussion:

On the basis of the virus report from Dr. Chen, the Italian semen-inseminated queens were released from quarantine by APHIS in late July 2012. The queens will be overwintered in WA and ID and in Spring 2013 will be used to produce *A. m. ligustica* hybrid virgin daughter queens that will be inseminated with additional imported/cryopreserved *A. m. ligustica* germplasm (to be reported in next reporting period).

The previous (2011) importations of *A. m. carnica* and *A. m. caucasica* semen resulted in overwintered queens that were maintained and used for selective breeding in 2012 (during this reporting period). These stocks are currently being tested and released (see 2012 complimentary report of SWC). Overall, the Italian honey bees exhibit behavioral

characteristics of primary apicultural interest to commercial pollinators in the United States, including docility, high rates of colony growth and good honey production.

This report represents the first significant progress toward the improvement of US honey bee populations based on the importation of novel honey bee genetic diversity widely accessed by the bee breeding industry. Additional funding provided by the Almond Board for 2012-2013 (to be discussed in the next reporting period) will support continued collection and additional importations of semen from Old World sources in 2013. In addition, a major breakthrough in honey bee semen cryopreservation technology developed in our laboratory (Hopkins et al., *In Press*) was used to cryopreserve aliquots of all semen collected in 2011 and 2012. In this reporting period, we utilized some cryopreserved semen to continue to make backcrosses with 2011-collected *A. m. caucasica* semen. *A. m. caucasica* is a subspecies that was previously maintained in the US, but has been considered "lost" for the past 20 years or more. The combined factors of 2010 and 2011 semen importation, careful maintenance of progeny colonies *in vivo* and the 2012 use of cryopreserved semen has allowed us to reconstruct a line of *A. m. caucasica* that contains 90% Old World germplasm. By 2013, we anticipate release of this previously available subspecies to US beekeepers.

The ability to cryopreserve semen, coupled with the established USDA-APHIS/WSU permit protocol for honey bee germplasm importation, now provides the opportunity for the development of a honey bee genetic repository in the United States. Such a repository would allow practical permanent storage of genetic material for subsequent breeding use, much as has become routine in other animals of agricultural significance (dairy and meat cattle, sheep, swine, horses, etc.). The Almond Board funding received by WSS for honey bee germplasm collection efforts has laid the groundwork for future requests to granting agencies to support the establishment of a permanent honey bee germplasm repository.

Research Effort Recent Publications:

No specific publications derived from the 2011- 2012 Almond Board award have been forthcoming. However, a related paper in the area of cryopreservation is currently in Press (Hopkins et al, *In Press*). Another manuscript *in prep* relates to recent advances in "above-freezing" long term storage of honey bee semen. In July 2012, we successfully obtained worker offspring from a queen that had been instrumentally inseminated with semen stored at 14C for 15 months and a paper describing this research is *in preparation*. Our 2012 collecting trip also included the collection of fresh semen for immediate use (within two weeks) and a stock of cryopreserved material for future use and to serve as original contributions to a honey bee germplasm repository. WSS and colleagues for the American Bee Journal are preparing a manuscript highlighting the collecting trips and the genetic material resource.

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