Germplasm Collection, Cryopreservation and Use in Genetic Improvement of Honey Bees

Project No.: **10-POLL7-Sheppard**

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Project Cooperators and Personnel:

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Objectives:

1) Import semen that will incorporate additional sex allele diversity into US honey bee populations; select and propagate honey bee stocks for industry that exhibit disease and parasite resistance, improved overwintering ability and cool season pollination behavior
2) Work toward establishment of a honey bee germplasm repository in the US to support future bee breeding; provide a possible genetic reserve for conservation efforts and genetic exchange with other repositories
3) Provide genetic material to CA queen producers for selection of stocks with traits best suited to the managed pollination of Almonds, including rapid population build-up under feeding management, heavy early spring brooding to stimulate pollen collection, and appropriate disease and pest resistance

Interpretive Summary:

This document represents the final progress report for an initial award of Almond Board ($5,000) to W.S. Sheppard to assist with expenses necessary to import novel honey bee germplasm. Honey bee germplasm (semen) from Old World original source populations in the Caucasus Mountains was collected in July of 2010 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 were maintained over the winter (2010-2011) in WA, ID and CA and are being made available to commercial queen producers to enhance the diversity of US honey bee populations. This project provides a mechanism for future introduction 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.

Materials and Methods:

In 2010, Walter Sheppard traveled to the Republic of Georgia and made a significant collection of *A. m. caucasica* semen from two disparate areas of the Caucasus Mountains. The primary areas sampled were Mestia, in the western Caucasus and Pasanauri in the central Caucasus (east of the South Ossetia region). Semen was collected from apiaries in these regions and then returned to the US in July 2010 under a USDA-APHIS hand carry permit awarded to WSS. California Queen producer collaborators had pre-shipped virgin queens from US domestic Carniolan stocks to WSU and several dozen of these virgin queens were inseminated with the imported Caucasian honey bee semen. Ms. Sue Cobey conducted the instrumental inseminations and also served as primary industry liaison 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. The collection and introduction into the US of genetic material derived from endemic honey bee populations has been ongoing 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 Caucasian semen-inseminated queens were released from quarantine by APHIS in August 2010. The queens were overwintered in CA, WA and ID and in Spring 2011 were used to produce *A. m. caucasica* hybrid virgin daughter queens that were inseminated in July 2011 with additional imported *A. m. caucasica* germplasm (to be reported in next reporting period). Overall, the Caucasian honey bees exhibited behavioral characteristics that will be of apicultural interest in the United States, including docility, cool-temperature activity 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 that can be accessed by the bee breeding industry. Additional funding by the Almond Board (to be discussed in the next reporting period) allowed a second year of collection in the Caucasus region and additional importations of semen from *A. m. carnica* in July 2011. In addition, a major breakthrough in honey bee semen cryopreservation technology occurred in our laboratory in 2010 with the production of sequential queens from cryopreserved semen (Hopkins et al., *In Press*). The ability to cryopreserve semen, coupled with the established USAD-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 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 $5,000 award of 2010-2011 Almond Board funding have been forthcoming. However, a related paper in the area of cryopreservation is currently in Press (Hopkins et al, *In Press*). Our 2011 collecting trip 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. A manuscript highlighting the collecting trips and the genetic material resource is being prepared by Walter Sheppard and colleagues for the American Bee Journal. In keeping with Almond Board policy, the manuscript draft will be submitted to the Almond Board **prior** to journal submission.

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