Honey Bee Stock Improvement Program: Importation, Preservation and Utilization of Honey Bee Germplasm

Project No.: **13-POLL7-Sheppard/Cobey**

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Objectives:

1. Continue to develop a cooperative industry/university bee breeding program to incorporate genetic material from imported honey bee germplasm into domestic breeding stocks and to implement practical programs to evaluate and maintain these stocks.
2. Continue the collection and importation of honey bee germplasm from endemic populations of European honey bees to enhance genetic diversity of domestic honey bee breeding stocks.
3. Implement cryopreservation of all imported 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.
4. Collect and cryopreserve honey bee germplasm from domestic, commercial bee breeding programs for future use by queen breeders.
5. Continue to offer and expand specialized Technology Transfer short courses.

Interpretive Summary:

Fundiing from the Almond Board to W.S. Sheppard/S.W. Cobey has assisted with expenses necessary to import and disseminate novel honey bee germplasm. Honey bee germplasm (semen) from Old World original source populations in Turkey and Georgia was collected in June of 2014 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 (2014-2015) in WA, ID and CA and made available to commercial queen producers in Spring 2015 to enhance the diversity of US honey bee populations. Previous germplasm (collected and used for insemination in 2013) was maintained in colonies in a similar manner and distributed in 2014. Through ongoing and future introductions of honey bee germplasm, this project provides a mechanism for the genetic improvement of commercial

honey bee populations, a critical resource to pollinate almonds and other agricultural crops. In addition, a cooperative bee breeding program has been established with members of the California Bee Breeders Association. The purpose of the program is to develop a sustainable, self-supporting, cooperative industry/uUniversity honey bee stock maintenance program and to incorporate the germplasm importations into domestic honey bee breeding stocks.

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 of bee breeders to select for resistance to *Varroa* and other pests and diseases.

Materials and Methods:

In 2014, Walter Sheppard (WSS), Sue Cobey (SWC) and Brandon Hopkins (BKH) traveled to Turkey and made a number of collections of semen from the subspecies *Apis mellifera caucasica* sourced from distinct locations in the Turkish Caucasus Mountains. WSS and BKH then continued the collection of Caucasian honey bee germplasm in the Republic of Georgia from apiaries in the region around Kutaisi, Georgia (Objective 2). In addition to fresh semen taken for immediate use upon return to the US, aliquots of a number of semen samples were also cryopreserved in liquid nitrogen for subsequent use in breeding (Objective 3). During this trip novel above-freezing storage techniques developed by our laboratory were employed that permitted extended times for insemination with fresh semen upon our return to the US.

Collected germplasm (semen) was returned to the US in late June 2014 under a USDA-APHIS hand carry permit awarded to WSS. We had previously produced virgin honey bee queens of our Washington State Univeristy (WSU) Caucasian hybrid lines, and over 60 queens were inseminated by SWC with "fresh" imported pure Caucasian honey bee semen. 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* (Caucasian).

In 2014, we also traveled to Baton Rouge, LA and cryopreserved a significant collection of the germplasm from the Russian Honey Bee Breeders Association (RBBA). This collection represents a major genetic "safety net" for this domestic honey bee population that was originally imported and selected by USDA-ARS to have improved mite tolerance (Objective 4).

The cryopreserved material from both domestic and imported sources is maintained at the WSU Honey Bee Germplasm Repository and this isfunded by Project Apis M.

Results and Discussion:

On the basis of the virus report from Dr. Chen, the queens inseminated with 2014-collected Caucasian honey bee semen were relatively free of virus (only black queen cell virus and deformed wing virus were detected). A Fall 2014 release of these inseminated queens from quarantine by USDA-APHIS is pending. The queens will be overwintered in WA, CA and ID and in Spring 2014 will be used to produce *A. m. caucasica* hybrid virgin daughter queens that will be inseminated with additional imported/cryopreserved *A. m. caucasica* germplasm (to be reported in next reporting period). It is expected that imported Caucasian honey bee germplasm will be used in domestic commercial queen production by at least 2 CA queen producers in 2015. Overall, Caucasian honey bees exhibit behavioral characteristics of apicultural interest to beekeepers that overwinter in northern and high altitude locations in the United States, including docility, high rates of colony growth during the spring and good honey production. Another characteristic of this subspecies is considerable usage of propolis (collected plant resins), a material that has recently been shown to improve colony level immune function.

We also note that further progress on above-freezing storage, cryopreservation and utilization of cryopreserved material was made during this reporting period. We implemented a method that uses a small battery powered Peltier system to maintain fresh semen at 14° C during the collecting process and subsequent travel and storage. With this system we are now able to get two generations of progeny (offspring) from fresh imported semen compared to the previous limit of one. Fertilized offspring from the queens of the initial cross were grafted and used to produce a new generation of virgin queens that were subsequently inseminated with fresh semen. As a result, the most recent crosses have queens that represent the 5th backcross from the Caucasian semen (although one natural mating for 2013 winter bees is included) and our current Caucasian stocks contain more than 90% genetic material from *A. m. caucasica.* This material is entering the US domestic honey bee population through a cooperative honey bee breeding program established with domestic queen producers in CA (Obj. 1 & 4). In 2015, we anticipate that production queens (open-mated daughters of our selected inseminated stock) will be available to the general population of beekeepers. In 2014, we also conducted a beginner's beekeepers course and a queen rearing course for beekeepers to help extend knowledge of the basic principles of selection and breeding in honey bees (Obj. 5). These courses were fully booked in 2014 and will be repeated in 2015. In addition, in 2013-2014 WSS, SWC, BKH and graduate students from the Sheppard Lab made numerous presentations to beekeeping associations in the western US (CA, OR, WA, ID) describing the research effort being supported by the Almond Board.

We report here significant progress toward the improvement of US honey bee populations based on the importation of novel honey bee genetic diversity widely accessible by the bee breeding industry. Funding provided by the Almond Board for 2013-2014 supported continued collection and additional importations of semen from Old World sources. The ability to cryopreserve semen, coupled with the established USDA-APHIS/WSU permit protocol for honey bee germplasm importation, has provided material that is maintained in the first honey

bee genetic repository in the United States. Such a repository will 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 mission of the WSU Honey Bee Germplasm Repository is to maintain original source population genetic stocks imported under Almond Board funding, to conserve "top-tier" genetics of existing US commercial stocks and other specific lines of honey bees submitted by other research laboratories and queen producers.

The Almond Board funding received by WSS and SWC for honey bee germplasm collection efforts continues to lay the groundwork for requests to granting agencies to support the establishment of a permanent honey bee germplasm repository. In 2014, we received funds from the Washington State Tree Fruit Research Commission (WSTFRC) to initiate a collection of honey bee germplasm from a subspecies of honey bee (*Apis mellifera pomonella*) that co-evolved with apples in the Tien Shan Mountains of Central Asia. The success of the Almond Board funded honey bee germplasm research and outreach efforts by our research group was a critical prelude to both growing acceptance of the new technology (RBBA project above) and to increased consideration of improved honey bee genetics (WSTFRC-funded project above).

Research Effort Recent Publications:

No specific publications derived from the 2013- 2014 Almond Board award have been forthcoming. A manuscript *in prep* relates to recent advances made in our laboratory on "above-freezing" long term storage of honey bee semen. In keeping with Almond Board policy, manuscript drafts will be submitted to the Almond Board prior to journal submission.

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