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

Project No.: 12-POLL7-Sheppard/Cobey

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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 annual report for an award of the Almond Board to W.S. Sheppard/S.W. Cobey 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 2013 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 (2013-2014) in WA, ID and CA, and will be made available to commercial queen producers in Spring 2014 to enhance the diversity of US honey bee populations. Previous germplasm (collected and used for insemination in 2012) was maintained in colonies in a similar manner and distributed in 2013. 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 used to

pollinate almonds and many 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/University 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-US origins, the US National Plant Germplasm Respository 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 2013, Sheppard, Cobey, Brandon Hopkins, and Jackie Park-Burris traveled to Italy and made a number of collections of semen from the subspecies *Apis mellifera ligustica* from distinct locations in Reggio-Emilia (Bologna), including commercial queen producers and a Federal honey bee laboratory charged with evaluating Italian honey bee genetic diversity. Given her experience with commercial Italian honey bee queen production, Park-Burris assisted with the evaluation and selection of Italian stocks that were taken for semen collection in 2013 and continues to work closely with the WSU program in her role as a member of the California Bee Breeders Association. 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.

Collected germplasm (semen) was returned to the US in late June 2012 under a USDA-APHIS hand carry permit awarded to Sheppard. California Queen producer collaborators had pre-shipped virgin honey bee queens of US "domestic Italian" stocks to WSU and over 80 queens were inseminated with the fresh imported Italian honey bee semen obtained in the previously described trip. Cobey 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: *A. m. ligustica* (Italian), *A. m. carnica* (originally derived from the Alps), and *A. m. caucasica* (originally derived from the Caucasus Mountains).

Results and Discussion:

On the basis of the virus report from Dr. Chen, queens inseminated with 2013-collected Italian honey bee semen were relatively free of virus (black queen cell virus was detected in only one sample). A Fall 2013 release from quarantine by APHIS is pending. The queens will be overwintered in WA, CA and ID, and in Spring 2014 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). It is expected that imported germplasm will be used in domestic commercial queen production in 2014 (Park Burris – personal communication). Overall, 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.

We also note that further progress on cryopreservation and utilization of cryopreserved material was made during this reporting period. We successfully thawed semen from collections made in 2011 in the Caucasus Mountains and used it to perform backcrosses within the WSU Caucasian honey bee breeding program. (Caucasian honey bees were previously used by US beekeepers, but have been unavailable for decades). Fertilized offspring from the queens of these crosses were grafted and used to produce a new generation of virgin queens that were subsequently inseminated with fresh semen. As a result, these queens represent the 3rd sequential backcross from the Caucasian semen and contain more than 82% genetic material from *A. m. caucasica*. This material is currently entering the US domestic honey bee population through a cooperative honey bee breeding program established with domestic queen producers in CA (Objective 4 above). By 2014, we anticipate that production queens (open-mated daughters of our selected inseminated stock) will be available to the general population of beekeepers. In 2013, we also conducted several queen rearing courses for beekeepers to help extend knowledge of the basic principles of selection and breeding in honey bees. These courses were both fully booked in 2013 and will be repeated in 2014. In addition, Sheppard, Cobey, and Hopkins 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 2012-2013 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, 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 Sheppard for honey bee germplasm collection efforts laid the groundwork for future requests to granting agencies to support the establishment of a permanent honey bee germplasm repository. In 2013, we received funds from Costco and

Project Apis m. to initiate a honey bee germplasm facility at WSU. This facility will maintain original source population genetic stocks imported under Almond Board funding, “top-tier” genetics of existing US commercial stocks, and other specific lines of honey bees submitted by other research laboratories and queen producers.

Research Effort Recent Publications:

No specific publications derived from the 2012- 2013 Almond Board award have been forthcoming. However, a related paper in the area of cryopreservation was published in 2012 (Hopkins et al., 2012). Another manuscript *in prep* relates to recent advances made in our laboratory on “above-freezing” long term storage of honey bee semen. Our 2013 collecting trip included the collection of both 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. In keeping with Almond Board policy, manuscript drafts will be submitted to the Almond Board prior to journal submission.

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