

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

Project Leaders: Susan W. Cobey and Walter S. Sheppard

Dept. of Entomology, Washington State University, Pullman, WA 99164

Project Cooperators: Brandon K. Hopkins, Washington State University; Marla Spivak, University of Minnesota; Judy Chen, USDA-ARS Bee Research Lab

Objectives

- Continue collection of germplasm from endemic populations of European honey bees
- Implement cryopreservation of representative samples of all collected honey bee germplasm for long term breeding use.
- Continue a selective breeding program to evaluate and improve introduced stocks and hybrids under US conditions, screening for resistance to pests and diseases
- Develop a cooperative Industry/University based program to disseminate imported honey bee germplasm and assist in the evaluation and maintenance of desirable breeding stocks
- Continue and expand specialized Technology Transfer short courses.

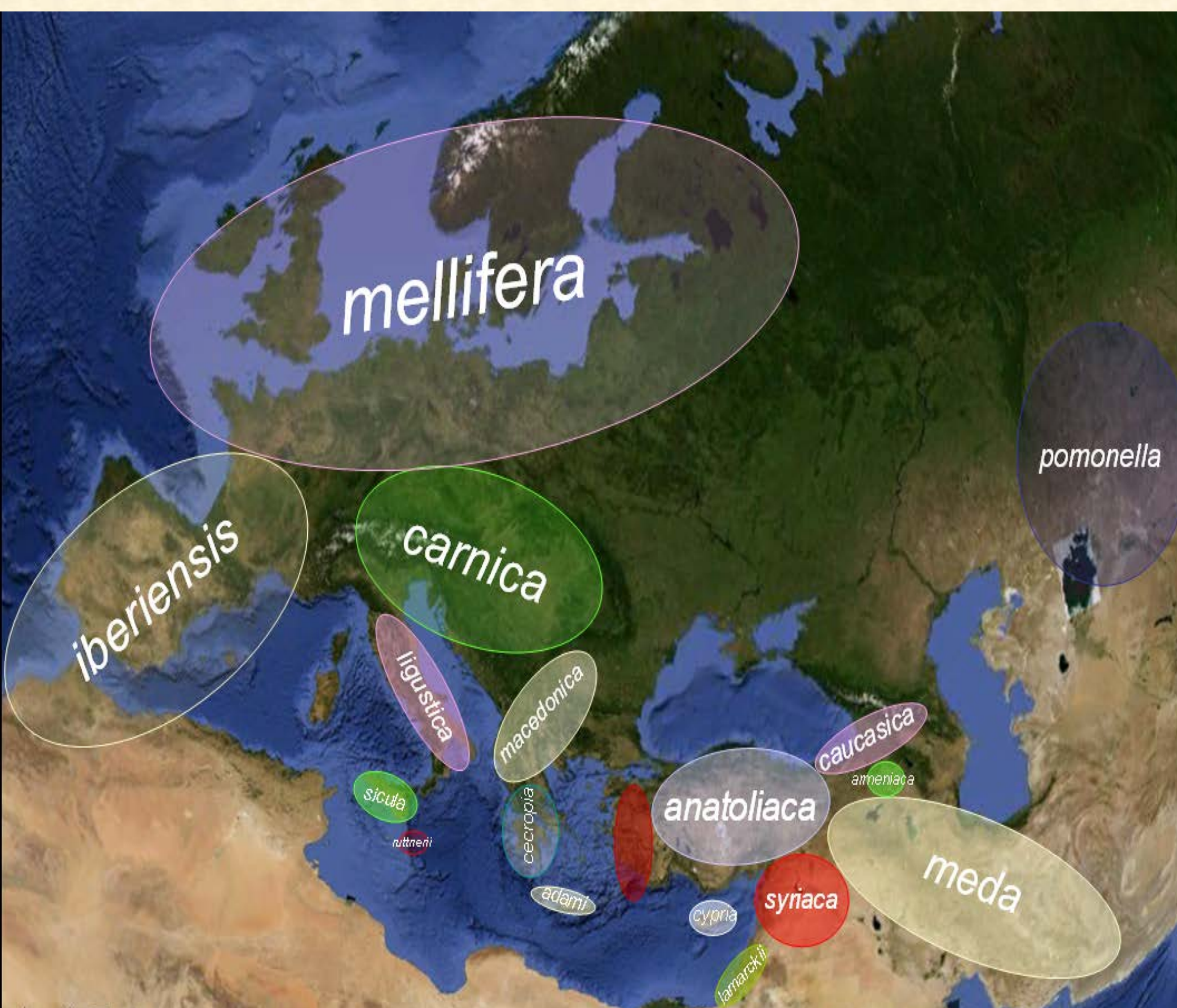
Background

Recent declines in honey bee populations and increasing challenges to maintain colony health are of concern to both beekeepers and growers of crops needing pollination services. Amid widespread research on honey bee health issues, there remains a notable lack of research directed toward the genetic improvement of honey bees.

There is a strong queen production industry in the US, but queen producers rely primarily on populations of bees established during the major period of importation between 1860 and 1922 (when the Honey Bee Act restricted importation of bees). While importation of honey bee germplasm effectively ceased after 1922, feral European honey bee populations in some southern states served as supplementary sources of genetic variation for breeding operations. However, the arrival of parasitic Varroa mites in the US in 1987 led to a major decline in US feral honey bee populations. The genetic effect of "bottlenecks" associated with importation and parasite-driven population losses includes a reduction in the amount of honey bee genetic diversity available to queen breeders.



The most practical means for honey bee germplasm entry to US is through collection of semen under permit and use of instrumental insemination for propagation of the genetic material



The Western honey bee is native to the Old World and three subspecies, *Apis mellifera ligustica*, *A. m. carnica* and *A. m. caucasica* are being sampled for germplasm as part of this project. (from L. Garnery)

Recent success of our laboratory in developing cryopreservation methods for bee semen, the establishment of a USDA-APHIS approved quarantine apiary at Washington State University for germplasm introductions and collaboration with California cooperators and the Bee Informed Partnership Tech-Transfer Teams provides new opportunities for bee breeding. With cryopreservation, honey bee semen can be preserved, stored and used in a matter of weeks, months or years through instrumental insemination of queens.



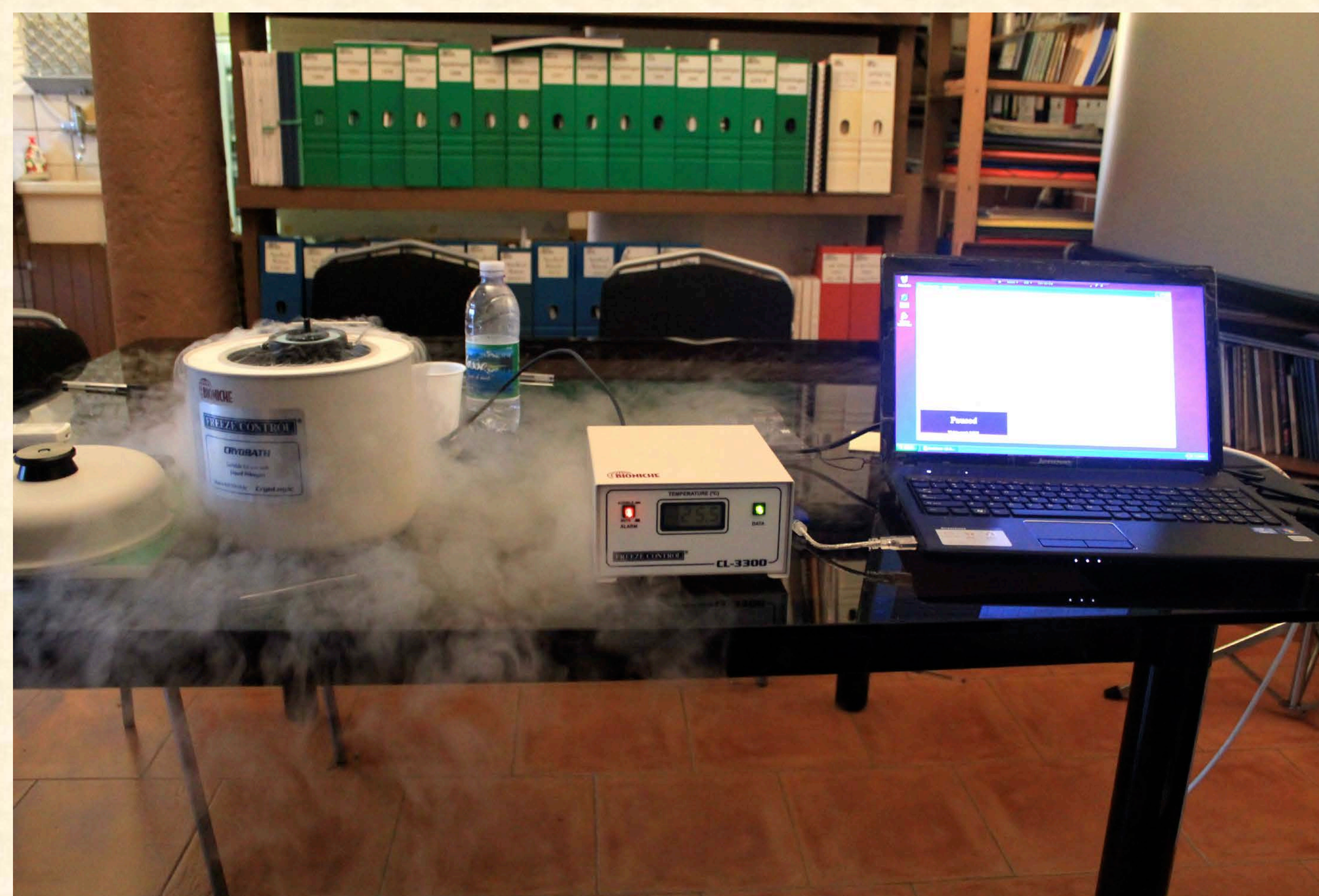
Device to assist in collecting *A. m. ligustica* drones



A collecting site for *A. m. ligustica* in Central Italy, 2012

In 2012 we (BKH, SWC, WSS) traveled to Italy and made significant collections of *A. m. ligustica* semen from a number of apiaries in the central Piedmont and the Reggio-Emilia (Bologna) area. Honey bees of this subspecies constitute the basis of current US "Italian" honey bees, the most widely used honey bee strain for managed pollination and honey production in the US. Interestingly, the Bologna region represents the original Italian source location for initial US importations of honey bees from Italy, made in 1860.

Semen from all sampling locations was collected for both fresh use and cryopreservation and returned to the US under a USDA-APHIS hand carry permit. Collaborating California queen producers had pre-shipped virgin queens from US domestic stocks to Pullman, WA and these were inseminated with imported fresh honey bee semen. Aliquots of this semen were concurrently supplied to Dr. Judy Chen of the USDA-ARS Bee Research Laboratory in Beltsville MD for virus determination. The collection and re-introduction of genetic material from source populations of historically introduced honey bee subspecies provides significant additional genetic resources for US bee breeding. Maintaining adequate genetic diversity in breeding stocks of bees is highly important, as queen breeders strive to select for disease and parasite resistance in bee stocks and to reduce reliance on chemotherapeutic agents.



Cryopreservation of *A. m. ligustica* honey bee semen in Reggio Emilia, Italy



Council for Research and Experimentation in Agriculture – Reggio Emilia Laboratory for Apicultural Research

Importation and Preservation of honey bee germplasm. Why?

1. Enhance genetic diversity of US honey bee populations
2. Provide new "raw material" for bee breeding efforts
3. Provides a means to preserve "top-tier" honey bee genetics



A frame of queen cells ready to place into mating nuclei



Queen mating apiary at WSU's Smoot Hill Ecological Station

This report chronicles progress toward the genetic improvement of US honey bee populations, based on the importation of novel honey bee germplasm from original source population locations. Funding by the Almond Board supported collection of Italian honey bee germplasm in 2012. Current US populations of managed honey bees are predominantly derived from 19th century importations of Italian bees.

We also applied cryopreservation technology developed at WSU to preserve aliquots of all semen collected in 2012 in liquid nitrogen for placement in a pilot germplasm repository. The combination of cryopreservation technology and an existing USDA-APHIS/WSU permit protocol to import honey bee germplasm, provides means to establish a honey bee genetic repository in the United States. A genetic repository will permit practical permanent storage of honey bee genetic material for subsequent use, as has become routine in programs supporting other animals of agricultural significance (dairy and meat cattle, sheep, swine, horses, etc.). In addition to imported genetic material, a germplasm repository provides a means for bee breeders to cryopreserve superior or "top-tier" genetics for later use; effectively allowing for breeding through "time and space". Almond Board funding support for honey bee germplasm collection, preservation, evaluation and distribution is a significant contribution toward the genetic improvement of honey bees.

Queen breeding and instrumental insemination Technology Transfer courses were offered in 2012 and will be repeated in 2013. Development of a pilot program with commercial beekeepers is currently underway to evaluate, maintain and distribute novel genetic material from the Honey Bee Stock Improvement Program to the beekeeping industry.



A. m. ligustica brood pattern



A. m. ligustica queen

Research Effort Recent Publications:

No specific publications based on the 2012 Almond Board funding were published. A manuscript highlighting the collecting trips and the genetic material resource is being prepared for the American Bee Journal/Bee Culture magazines. In keeping with Almond Board policy, manuscript drafts will be submitted to the Almond Board prior to journal submission.

Relevant References :

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, W.S. 2012. Sequential generations of honey bee (*Apis mellifera*) queens produced using cryopreserved semen. *Reproduction, Fertility and Development*. Doi:10.1071/RD11088

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. (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.



Our Italian collaborator, Professor Raffaele Monaco of the University of Bari, advises a local beekeeper