Honey Bee Stock Improvement Program

Project No.: **09-POLL4-Cobey**

Project Leader: Susan Cobey
Department of Entomology
UC Davis
Harry Laidlaw Honey Bee Research Facility
367 Briggs Hall
Davis, CA 95616
(530) 754-9390
fax: (530) 754-7757
swcobey@ucdavis.edu

Project Cooperators and Personnel:

Dr. John Pollard and Dr. Claire Plante, Genesafe Technologies Ltd., Guelph, Canada
Dr. Steve Sheppard, Washington State University, Pullman
Dr. Michelle Flenniken, UC San Francisco
Dr. Marla Spivak, University of Minnesota
Dr. Jeff Pettis and Dr. Judy Chen, USDA Bee Research Laboratory, Belstsville, Md.
Elizabeth Frost, Department of Entomology, UC Davis

Objectives:

1. To enhance genetic diversity of domestic honey bee stocks through importation and selection programs to increase the level of resistance to pests and diseases.
2. To develop commercially viable reproductive technologies and protocol for the safe international exchange of honey bee germplasm.
3. To provide training in techniques to advance the development, establishment and maintenance of productive commercial breeding stocks.

Interpretive Summary:

Enhancement of Domestic Stocks

Honey bee semen of *Apis mellifera ligustica* from Italy and *A.m. carnica* from Germany were imported in 2008 and 2009 under USDA-APHIS (Animal Plant Health Inspection Service) permits and mated to virgin queens from proven commercial stocks. The resulting colonies were established in approved quarantine areas in Washington State. The resulting colonies of New World Carniolan crossed with German *carnica* were released from quarantine and established at UC Davis during the summer of 2008. Evaluations showed expression of hybrid vigor, a high degree of hygienic behavior, good productivity and gentle temperament. A limited release of daughter queens was made to participating California queen producers. Daughter virgin queens were also

backcrossed to *carnica* semen imported in 2009; the resulting colonies are currently in quarantine.

Colonies headed by queens inseminated to the 2008 imported Italian semen were over-wintered in the Washington quarantine area because the semen tested positive for virus. In progeny testing of these colonies, 75 % showed no transmission of virus. Most colonies did not survive the severe winter for which this stock is not well adapted, therefore only three colonies released survived to spring of 2009. These were moved to California and daughter virgin queens were propagated and mated to the 2009 Italian semen import. The resultant colonies are currently in quarantine.

We also plan to import semen of *A.m. caucasica* from Turkey. Traces of this subspecies are detectable in the US gene pool, but have been largely lost. Valuable contacts and future plans are being developed.

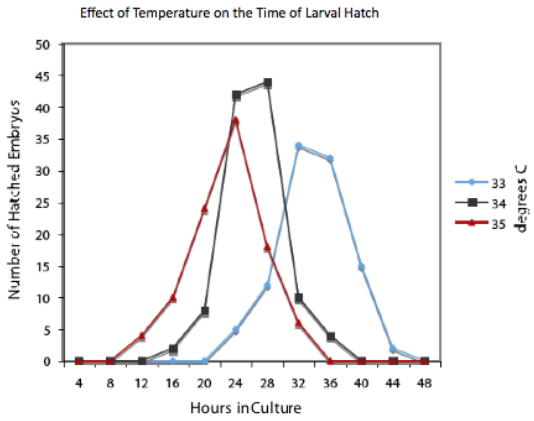
Reproductive Technology Development

Honey bee embryos are good candidates for transfer because they provide a complete genetic package and the queen has a high rate of production. We developed a new method for the manipulation of embryos improving the rate of success and to allow for their isolation, pathogen testing, long distance transport, *in vitro* hatching and subsequent grafting for queen production. Micro-forceps were modified by the application of flexible micro-bore tubing to the distal pincers. The apical end of individual embryos are gently grasped between opposing segments of the flexible tubing and then lifted to separate their glued basal end from the comb. The embryo transfer forceps are pictured in **Figure 1**. Using this technique eggs are transferred in isolation to plastic culture dishes. The embryos maintain their upright position, and naturally re-glued to the dishes. This eliminates the need maintain the attached beeswax base which may carry pathogens.

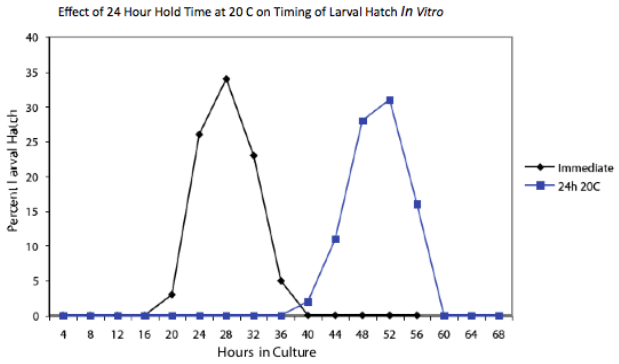
Figure 1. Embryo transfer forceps with mircobore tubing and egg.

To understand the limits of this technique, we explored the effects of temperature on egg hatch for long distance transport. The honey bee embryo stage is 72 hours, and is cooling tolerant at 50-54 hours post-oviposition. To obtain this stage queens were confined on comb with access to worker bees for a timed period. Embryos were transferred in groups (n=100) to sterile plastic culture dishes and naturally re-glued in a standing position on plastic dishes and then placed into 35^{o}C incubation with 95% humidity for 32 hours. Selected *in vitro*-hatched larva, demonstrating spiracular movement, were grafted into queen cell cups and transferred to cell builder colonies and reared to queens.

The temperature effects on the time of larval hatch were assessed to determine the limits and timing of egg incubation. A one degree change would slow or speed larval hatch by several hours, as presented in **Graph 1**. Embryos held at 20º C for 24 hours shutdown, but would recover and hatch with a temperature increase, as presented in **Graph 2.**



Graph 1. Effect of Temperature On the Time Of Larval Hatch.



Graph 2. Effect of 24 Hour Hold Time at 20º C on Timing of Larval Hatch *In Vitro*

Grafted larvae were assessed for acceptance and for queen emergence. Results of the embryos cultured in 2008 and 2009, demonstrated that 94% and 92% of transferred embryos hatched *in vitro* within 32 hours of culture. Of the larvae grafted for queen production, 42% and 40% were accepted and emerged as morphologically normal queens. These queens were instrumentally inseminated and introduced into colonies with normal acceptance rates of 86% and 78% respectively. The mean rate of initiation of oviposition was 5.5 and 6.0 days, as presented in **Table 2**.

Viability Testing of Transferred Embryos

Results of the *In Vitro Culture* of Transferred Embryos and Grafting of *In Vitro* Hatched Larvae

Year	Total Embryos Cultured	Hatched (%)	Total Larvae Grafted	Larvae Accepted	Queens II'd	Accepted	Day of Oviposition
2008	998	941 (94%)	570	239 (42%)	107	92 (86%)	5.5
2009	1734	1595 (92%)	639	250 (40%)	137	106 (78%)	6

Table 2. Viability Testing of Transferred Embryos

To manage the safe movement of eggs, we propose an Embryo Transfer System outlined in **Figure 2**. The queen is confined to obtain known age eggs. The eggs are transferred at 50 to 54 hours into sterile 96 well plates maintained at 20ºC for 24 hours and then pressed directly into the comb of queenless and broodless cell builder colonies where the hatching eggs are immediately attended by nurse bees. In hive placement

eliminates the step of grafting small, delicate larvae 2 hours post-hatch and improved the acceptance rate of embryos to hatched larvae from 51% to 85%.

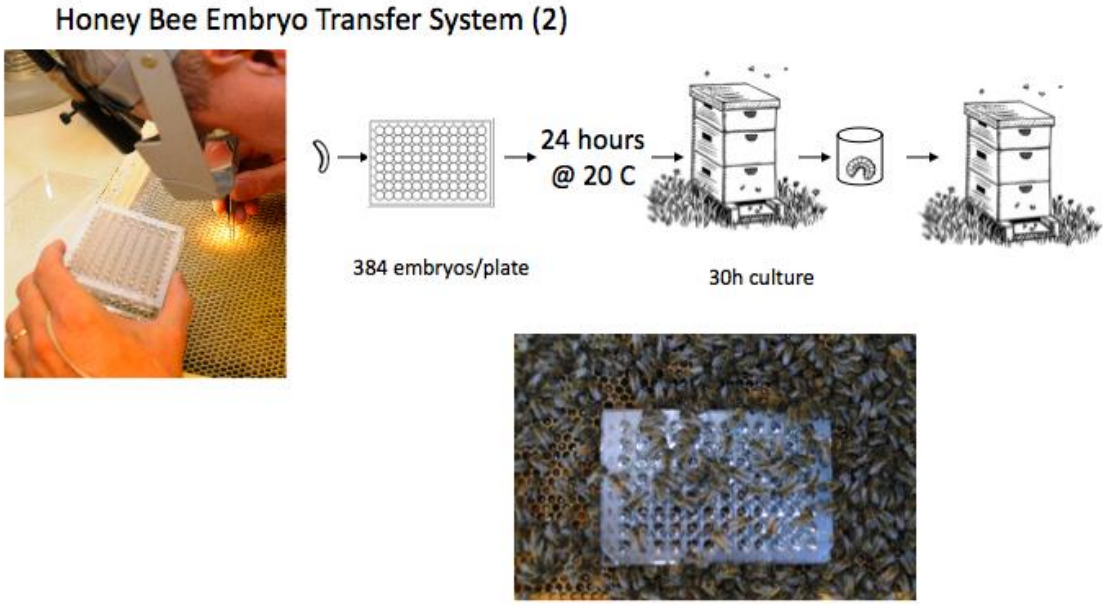


Figure 2. Honey Bee Embryo Transfer System 2. Well plates are pressed directly into the comb of queenless and broodless cell builder colonies.

The transmission of virus in honey bee germplasm remains a health risk question for international transport. In collaboration with Dr. Michelle Flenniken, we are testing a screening process using the Bee Pathogen Chip for the direct pathogen testing of donor colonies and gamete samples. Studying the virus profile of various aspects of colonies may provide insight into procedures to eliminate pathogens of bee gametes. This project is on-going with future plans to further develop and test importation protocols for safe and effective international exchange of honey bee genetics.

Technology Transfer

Three specialized beekeeping short courses were designed to provide the beekeeping community with the skills required to develop and maintain honey bee stocks. These classes will be offered annually. The 2008 and 2009 spring classes were well attended, beekeepers from across the country and abroad participated. Due to the high demand, a second queen rearing class and third insemination were scheduled in 2009. Plans are being made to expand the classes to meet the demand. The three courses offered are:

1. The Art of Queen Rearing, and tour of several commercial queen producers.
2. Instrumental Insemination of Queen Bees & Bee Breeding.
3. Advanced Techniques In Instrumental Insemination of Queen Bees.

References and Related Citations:

Collins, AM. 2002. Collection of honey bee eggs for cryopreservation. *J. Apic. Res..*41(3-4):89-95

Fuchs, S., & Schade, V. 1994. Lower performance in honey bee colonies of uniform paternity. *Apidologie, 24*, 155-168.

Jones, C.J., Myerscough, M., Graham, S., Oldroyd, B.P. 2004. Honey bee nest thermoregulation; Diversity Promotes Stability. *Science* 305, 402-404

Mattila, H. R., & Seeley, T. D. 2007. Genetic Diversity in Honey Bee Colonies Enhances Productivity and Fitness. *Science,* 317, 362-364.

Schiff, NM., & Sheppard, W. S. 1995. Genetic Analysis of Commercial Honey Bees from the Southeastern United States. *J.Econ. Entomol., 88*(5), 1216-1220.

Schiff, NM., & Sheppard, W. S. 1996. Genetic differentiation in the queen breeding population of the Western United States. *Apidologie, 27*, 77-86.

Sheppard, WS. 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.

Sherman, PW., Seeley, T. D., & Reeve, H. K. (1988). Parasites, pathogens and polyandry in social hymenoptera. *Am. Nat., 131*, 602-610

Taber, S. 1961.Forceps design for transferring honey bee eggs. *J. Econ. Entomol.* 54:247-250.

Tarpy DR, Seeley TD 2006 Lower disease infections in honeybee (*Apis mellifera*) colonies headed by polyandrous vs monandrous queens. 93: 195-199.

Seeley TD & Tarpy DR, 2007. Queen promiscuity lowers disease within honey bee colonies. *Proc. Royal Society of London,B.,* 274:67-72.

Wang D, Urisman A, Liu YT, Springer M, Ksiazek TG, Erdman DD, Mardis ER, Hickenbotham M, Magrini V, Eldred J, Latreille JP, Wilson RK, Ganem D, DeRisi JL. 2003. Viral discovery and sequence recovery using DNA microarrays. *PLoS Biology*.