Genetic Stock Identification Of Production Colonies Of Russian Honey Bees

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In 2008, the USDA-ARS Honey Bee Breeding, Genetics, and Physiology Laboratory (USDA-ARS HBB Lab) fully released the Russian honey bee stock to the Russian Honey Bee Breeders Association (RBBA). Each year, members of the RBBA are required to have their breeding stock genetically certified, to conduct tests of honey production, and assess pest and pathogen (Varroa mites, tracheal mites and *Nosema ceranae*) status.

When the stock was released the stock diversity was characterized and a suite of genetic markers was identified that would be used for later stock certification (Bourgeois and Rinderer 2009, Bourgeois *et al.* 2010). A summary of the procedures used to certify breeding stock is given below. The members of the RBBA often use different times and mating apiaries to produce the production queens that they use in honey production apiaries and market to other beekeepers. We were curious about the genetic nature of their production queens. Hence, in Autumn 2009 and Spring 2010, we asked beekeepers of the RBBA to submit samples of worker bees from their production queens to determine the level of Russian alleles in the production colonies.

A total of 5 of the 8 certified members of the RBBA submitted samples for this study. All bees were submitted as live bees and were frozen upon receipt. DNA was then extracted according to published protocols (Bourgeois *et al.* 2010). Russian and non-Russian alleles were identified as previously described in Bourgeois *et al.* (2010). Briefly, 8 individual bees per colony were genotyped with 12 microsatellite and 5 single nucleotide polymorphism (SNP) markers. These markers created a genetic fingerprint of each bee. After genotyping was completed, the data were visually inspected to identify bees that had "drifted" into the colony. This was done

using the identification of the queen's alleles. All bees that do not have one of the queen's two alleles for each locus were considered to have "drifted" and were eliminated from the analysis pool. This procedure is a component of the standard procedure used for testing certification samples for Russian honey bees.

After drifting bees were identified and removed from the data set, ONCOR software (http://www.montana.edu/kalinowski) was used to determine genetic stock identification. The software algorithm compared the genetic fingerprint of the test bees to the genotypes of bees that comprise a baseline sample of Russian and non-Russian honey bees from commercial operations throughout the U.S. The software provided the probability of assignment of each bee to either the Russian or Non-Russian group. The minimum acceptable threshold for assignment to the Russian group is held at 70% for each bee for stock certification. Because the production colonies are openly mated and are not held to the strict standards required for the propagation of breeder stock (i.e., isolated mating yards and strict control of drone source colonies), we would expect to see lower levels of Russian alleles in production queens. Dilution of the stocks' alleles is not uncommon in production yards where open mating and drift are likely to occur.

Overall, colonies had a mean probability of assignment to the Russian group of 0.66 ± 0.04. This compares favorably with a recent report of stock assessment in commercial operations (Spivak *et al.* 2009) using Minnesota hygienic bees. An assessment of commercial apiaries using the Minnesota Hygienic stock of honey bees 10 years post-release showed that 24-29% of sampled colonies exhibited the hygienic trait at a high enough rate to be considered as good potential breeder colonies (Spivak *et al.* 2009). In the Russian production colonies, 48% had an average assignment value of > 0.7 meaning that 48% of these colonies could serve as potential breeder colonies.

The variability in probability of assignment was high among colonies and among beekeepers (Figure 1). Stock assignments of individual colonies ranged from 0.95 to the Non-Russian group to 1.0 to the Russian group. SAS 9.2 was used for all data analyses. Group assignment values were consistent among beekeepers ($P > 0.05$) with all operations having a large degree of variation among colonies (Fig. 1). A comparison of the production colonies with certified breeder colonies for each beekeeper showed that breeder colonies have higher percentages of Russian alleles (0.94 ± 0.01; $P < 0.001$). This was expected, because the RBBA members must follow strict guidelines while setting up their mating yards. The production yards are not held to the same restrictions. However, RBBA members work only with Russian bees in their apiaries. The primary source of non-Russian alleles would be from nearby apiaries or feral colonies. In other words, some level of introgression of non-Russian alleles is expected in openly mated production colonies.

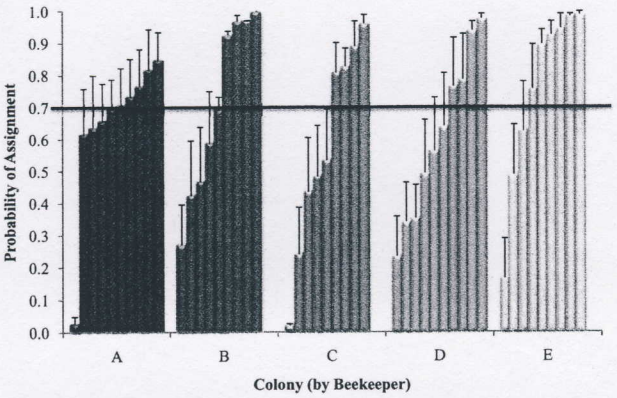

Figure 1. Genetic stock identification of production colonies of Russian honey bees. Bars represent mean ± S.E. for colonies from five Russian honey bee breeders. The horizontal line signifies the minimum assignment threshold for the certification of breeder stock that is used by the Russian Honey Bee Breeders Association.

The high level of Russian alleles in the production colonies is an indication that the bees should exhibit the characteristics of the USDA-ARS stock of Russian honey bees. Even in the presence of non-Russian alleles, the continuous selection that the Russian stock undergoes through activities of the RBBA should maintain the positive stock characteristics.

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