The Future of the MN Hygienic Stock of Bees is in Good Hands!

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In our December 2008 American Bee Journal article we described the history of the MN Hygienic stock and our plan to return to our original goal of encouraging beekeepers and queen producers to select for the hygienic trait from among their own colonies and stocks of bees.

The reasons for selecting bees for hygienic behavior are clear: hygienic colonies can defend themselves against American foulbrood, chalkbrood and *Varroa*. Here we show that three queen producers from Minnesota have successfully incorporated the hygienic trait into their operations. In fact, their naturally mated colonies are just as hygienic as the instrumentally inseminated breeder queens we had been providing them! This means that they can select and maintain the MN Hygienic stock on their own. We are extremely encouraged by the results of extensive testing we did on their colonies this past summer (2009). In effect, we can certify the probability that their queens will produce hygienic colonies. We sincerely hope this article encourages all queen producers to select for hygienic behavior and creates an open discussion on the future of trait certification in the US.

The Three MN Beekeepers: Darrel Rufer, Mark Sundberg, and Jeff Hull

These three Minnesota-based beekeepers have been raising queens from the University of Minnesota's Hygienic stock for over 10 years. They are all migratory beekeepers who move their colonies to the South every winter to raise queens and make divides. They successfully incorporated the hygienic trait into their operations because, over time, they requeened all of their colonies with daughters of highly hygienic queens. The daughter queens produced drones that carried the hygienic trait. Now when their new hygienic queens go on mating flights the majority of drones they encounter come from other hygienic colonies. We ensured that the original hygienic stock they received from the University was genetically diverse, and by looking at the solid brood patterns of their colonies, we are confident that the colonies are not inbred.

June 2009, after they transported their colonies back to Minnesota, we tested approximately 100 colonies across 3 or 4 apiaries in each operation for hygienic behavior using the freeze-killed brood assay (see Box 1). The time it takes the bees in a colony to remove freeze-killed pupae is correlated with how long it takes them to detect and remove disease and mite-infested brood from the colony. The faster a colony removes freeze-killed brood, the more resistant it is to American foulbrood, chalkbrood and *Varroa* mites. For more detailed information, including a printable poster with instructions, see the University of MN Bee Lab website at: http://www.extension.umn.edu/honeybees/.

There are two ways to interpret the results of the hygienic test. The strict measure is to consider only the percentage of freeze-killed brood that is completely removed within 24 hours **(% removed)**. Completely removed means there are no remains of the dead pupae in the cells after 24 hours. We use this strict test to choose breeder queens for the next generation of selection: only those colonies that completely removed ≥ 95% of the freeze-killed brood within 24 hours are chosen as breeders.

The liberal measure includes the pupae that are completely removed and those we call "partials" that are in the process of being uncapped and removed **(% removed + partials)**. From our experience, the brood that is partially uncapped and/or partially removed will be completely removed by 48 hours, so the liberal measure gives an approximation of how clean the test will be by the next day. We use the results of this liberal test to characterize the level of hygienic behavior in a population of colonies.

Comparison of Results with MN Hygienic Breeders and Unselected Stocks

The results of the liberal hygienic tests of the three MN beekeepers are shown in Table 1. For comparison, we also show results from hygienic tests on colonies at the Uni-

Table 1. Results of Hygienic Behavior tests among three groups of colonies: 1) colonies with instrumentally inseminated breeder queens from the MN Hygienic breeding program (tested over a 5 year period), 2) colonies with naturally mated queens produced by three MN beekeepers, 3) colonies from a study (Spivak and Reuter, 2001) in which 4th generation hygienic queens were naturally mated with unselected drones, and colonies with unselected queens mated with unselected drones.

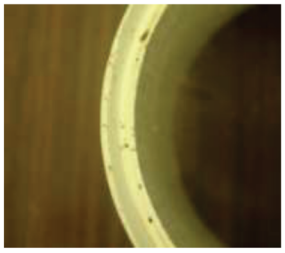
Liberal test results are shown as the average level of hygienic behavior for the groups of colonies, with different letters indicating a significant difference, and the percent of colonies that scored ≥ 95%. Strict test results show the percent of colonies that completely removed ≥ 95% of the freeze-killed brood within 24 hours, and that could therefore be selected as breeder colonies for the next generation.

		Liberal Test		Strict Test
Colony Source	Colonies tested	Average ± s.d. score % removed+ partials	% colonies scoring ≥95% removed+ partials	% colonies scoring ≥95% completely removed
1. MN Hygienic Breeders, University MN (2004-2008)	171	96% ± 8% a	75%	36%
2. MN Beekeeper 1 (2009)	118	96% ± 6% a	79%	29%
MN Beekeeper 2 (2009)	123	92% ± 11% a	63%	24%
MN Beekeeper 3 (2009)	87	92% ± 12% a	62%	24%
3. Hygienic Q + unselected drones (1999)	61	82% ± 18% b	38%	2%
Unselected Q + unselected drones (1999)	47	75% ± 19% c	13%	0%

Box 1. Performing a Hygienic Test:

Step 1. Open the desired colony and select a frame with sealed brood. Check a few cells for the age of the pupae. You want to freeze pupae that are midway through development: make sure you can see the eyes of the pupae; they can be white, pink or purple.

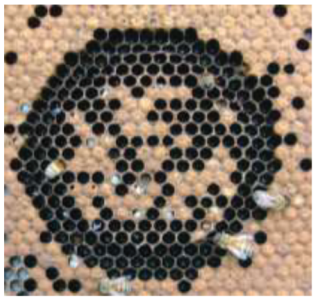
Step 2. With a twisting motion, press a 3" diameter tube into a patch of sealed brood down to the midrib of the comb. For the tube, we use PVC pipe with a groove cut using a 90° V-shaped metal router – see left picture below. Count and record the number of cells not sealed by wax. There are, on average, 160 cells encompassed by the tube. Subtracting the number of unsealed cells from 160 gives the number of sealed cells.

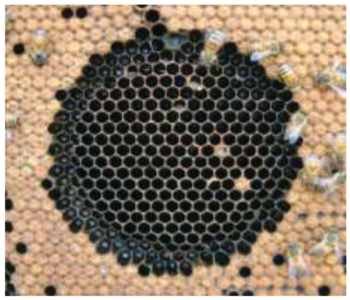


Step 3. Fill a 10 oz Styrofoam coffee cup with liquid nitrogen. To create a seal, pour approximately ¼" into the tube, wait until it bubbles off, then pour in the rest. Let the nitrogen evaporate and allow the comb to thaw until you can gently remove the tube without damaging the cells.

Step 4. Mark the frame so it is easy to identify (we use a colored tack to mark the frame and the side of the frame the test is on) and return the frame to the colony.

Step 5. In 24 hours retrieve the frame. Count and record the number of cells still completely capped and the number of cells the bees have begun to work on (referred to as partials), which range from cells starting to be uncapped to pupae chewed almost to the bottom of the cell. Below shows a test from a non-hygienic colony (left) with many capped cells and partials, and a hygienic colony (right) with just one capped cell and three partials.





To calculate the % removed and the % partials and removed use the following formulas:

$$\%\text{removed} = \frac{160 - \#\text{ unsealed cells at the start} - \#\text{ cells sealed at 24 hrs}}{160 - \#\text{ unsealed cells at the start}}$$

$$\%\text{removed} + \text{partials} = \frac{160 - \#\text{ unsealed cells at the start} - \#\text{ cells sealed at 24 hrs} - \#\text{partials at 24 hrs}}{160 - \#\text{ unsealed cells at the start}}$$

versity of Minnesota. The University colonies contained queens from the MN Hygienic stock that were reared and instrumentally inseminated at the University in the summer, and were wintered either in MN or in southeast TX. We show the tests for 171 inseminated queens from 2004 through 2008 that survived a winter.

To show the progress that has been made over the years, we also compared the tests of the MN beekeepers' colonies and the University of MN breeder colonies with those from a published study we ran 10 years ago, in 1999 (Spivak and Reuter, 2001). In that study, we compared colonies containing hygienic queens from the 4th generation of the breeding program that were naturally mated with unselected drones, to colonies with unselected queens mated to unselected drones (Table 1).

We show the average level of hygienic behavior for each group of colonies, using the results of the 24 hour liberal test. We also show the percentage of colonies that scored ≥ 95% for the liberal test. Finally, for the strict test, we show the percentage of colonies that could be selected as breeders for the next generation (i.e. those colonies that completely removed ≥ 95% of the freeze-killed brood within 24 hours).

Take Home Messages

1) The results of the tests using the liberal measure of hygienic behavior from colonies owned by the three MN beekeepers are similar to the results of the breeder queens in the MN Hygienic stock (refer to Table 1). If you purchase a queen from one of these beekeepers, there is 62%-79% chance that the queen will produce a hygienic colony (e.g., it will score ≥95% removed+ partials in 24 hours). This is a very high probability!
2) The strict measure is used to choose breeder queens for the next generation. The MN beekeepers had 24 - 29% of the tested colonies that could be used as breeders. Considering that each of these beekeepers had over 2,000 total colonies, they have many potential colonies to choose from as breeders! Also, these hygienic colonies are producing drones that carry the trait, ensuring that queens in the next generations mate with these males and carry on the trait.
3) The colonies with hygienic queens mated with unselected drones from the 1999 study were considerably less hygienic than the MN breeder colonies and the colonies owned by the MN beekeepers, by both strict and liberal measures of hygienic behavior. This shows that for colonies to be hygienic, the drones in the area where the queens mate should be from hygienic colonies as much as possible. Many hygienic queens sold in the US currently fall in this category: they are mated with unselected drones and on average, are not highly hygienic.

On the other hand, the colonies with hygienic queens mated with unselected drones from the 1999 study were notably more hygienic than the unselected queens mated with unselected drones. This shows that simply using hygienic queens helps! Hygienic queens produce hygienic drones for future matings!

Trait Certification?

By testing a number of colonies, we can certify the level of hygienic behavior in the stocks owned by the three MN beekeepers. Beekeepers can purchase a queen from these beekeepers knowing the probability that the queen's colony will be hygienic, which illustrates how certification of selected stocks or traits of bees might be carried throughout the US. We think beekeepers should be willing to pay a higher price for certified stock. The possibilities for the future are rich and varied. For example, once we have good field assays, we could certify other traits that help bees resist *Varroa* such as grooming. We plan to continue helping the MN beekeepers with stock selection to maintain the quality of their hygienic stock and to ensure they maintain sufficient genetic diversity within their operations.

As shown here, beekeepers can successfully incorporate the hygienic trait into their stock. Here beekeepers purchased instrumentally inseminated queens from the University of MN. It is important to note, however, that all stocks of bees carry the hygienic trait, and the use of inseminated queens as breeders is not necessary. We encourage all beekeepers to select their stocks of bees for hygienic behavior. We strongly emphasize the need for genetically diverse

stocks of bees in the U.S., and discourage the use of one or two stocks of bees, which could narrow our gene pool and lead to monocultures of bees. Choose your own colonies with desirable traits, such as high honey production, gentleness, good wintering ability and queen longevity. Perform the hygienic test on those colonies, and breed from the highest scoring ones. Requeen with the daughters and repeat each year with new breeder queens. It may take 4-6 years to saturate your area with hygienic drones, but you will see notable results! If you want to eliminate chalkbrood and AFB in your colonies, and reduce your *Varroa* mite loads, start your selection program for hygienic behavior today!

MN beekeepers (order of names randomized). All have limited availability of queens. They do not sell packages!!

Darrel Rufer - only orders for 50+ queens: (612) 325-1203

Mark Sundberg - large and small orders: (218) 721-5942, mdsund2000@yahoo.com

Jeff Hull – very limited availability, (218) 205-6426

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