Hygienic Behavior by Honey Bees From Far-eastern Russia

by LILIA I. DE GUZMAN[1], THOMAS E. RINDERER[1], J. ANTHONY STELZER[1], LORRAINE BEAMAN[1], GARY T. DELATTE[1], CHARLIE HARPER[2]

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Abstract

The rate of dead bee removal by Primorsky and domestic honey bees was compared using the liquid nitrogen technique. Results from two assays showed that Primorsky honey bees consistently removed more dead brood than the domestic colonies. For both assays, 41% of the Primorsky honey bee colonies tested were considered hygienic (≥95% dead bee removal). Only 21% of the domestic colonies showed the hygienic trait. No correlation between removal rate and adult bee population was observed.

KEYWORDS: Hygienic behavior/Primorsky bees/Far-eastern Russia/USA

INTRODUCTION

The hygienic behavior of honey bees has been recognized as an important mechanism of general resistance to their diseases and pests. Hygienic bees are able to detect, uncap and remove diseased or mite-infested brood. In *Apis mellifera*, this behavior was first observed as a resistance mechanism to American foulbrood (Rothenbuhler 1964a) and chalkbrood (Gilliam et al. 1983, 1988). *A. cerana* is effectively hygienic with *Varroa destructor* infested brood (Peng et al.1987a, Rath and Drescher 1990). *A. mellifera* is also hygienic with *V. destructor* infested brood, but at a much lower frequency (Peng et al. 1987b, Boecking and Drescher 1991, Boecking and Drescher 1992, Spivak 1996). The detection and removal of mite-infested pupae was also observed in the giant honey bee, *A. dorsata*, against *Tropilaelaps clareae* (Ritter and Ritter-Schneider (1988).

Because of many negative effects of using chemicals, propagation of honey bees with natural resistance to pests and diseases is highly desirable. Although hygienic behavior is an easily selected trait which contributes to this goal, only 10% of commercial honey bees in the United States are hygienic (Spivak and Reuter 1998a). The USDA, Agricultural Research Service (ARS), has released a stock of honey bees from far-eastern Russia (Primorsky) to the industry because of their ability to regulate populations of *V. destructor* in the colonies (Rinderer et al.1999, Rinderer et al. 2000, Rinderer et al. 2001, In Press). The full range of mechanisms of resistance to *V. destructor* employed by ARS Primorsky honey bees has not been fully explored. This study was conducted to evaluate the hygienic behavior of ARS Primorsky honey bees, since hygienic behavior may be part of their increased resistance to *Varroa* mites.

MATERIALS AND METHODS

The hygienic behavior of 29 ARS Primorsky and 19 domestic colonies of commercial stock was compared. All colonies had one deep and one shallow super and were set on pallets. Test colonies were located in two apiaries near Carencro, Louisiana.

The removal rate of colonies was determined by freezing a 3-inch diameter circular section of capped worker brood enclosing about 300 cells within the frame by using liquid nitrogen (Spivak and Reuter 1998b). Using a digital camera, test sections were photographed before the liquid nitrogen was poured. Test sections were also mapped in plastic sheets to facilitate identifying them after they were returned to the colonies. Brood frames with the frozen section were placed at the center of the brood nest of their respective colonies for 48 hours. The test sections were then photographed again. Examinations of photographs from before and after freezing and exposure of the test sections in colonies produced counts of the number of frozen cells with brood, and the number of frozen brood cells which remained capped. The numbers of cells that had traces of bee parts were determined from direct examination of the combs. The number and percentage of cells that were subjects of complete hygienic behavior were then calculated. Uncapped cells with any remaining traces of pupal parts were not counted as evidence of hygienic behavior.

Assays were begun on October 10 and November 1, 2000. The weather during the first assay was sunny, relatively cool (9-19°C) and there was no nectar flow. During the second assay, it was sunny, temperatures were higher (20-28°C), and goldenrod was supplying copious nectar and pollen. Two weeks prior to the second assay, all colonies were fed with sugar syrup (about 3 liters for each colony).

Colony strength was estimated during the first assay only. The numbers of deep frames covered by bees and filled with capped brood were estimated as described by Burgett and Burikam (1985). Although uncapped brood (eggs and larvae) was present in the colonies, it was not included in the estimation.

Data on brood removal were analyzed using ANOVA in a Split-plot design. Honey bee type, sampling date, and apiary site were modeled as fixed effects using Proc Mixed. Colony within type and site was modeled as random effect. Pearson's correlation coefficient was used to test the relationship between brood removal and the number of frames with adult bees present in the colonies (SAS Institute, 1997).

RESULTS

Brood removal - Overall, the ARS Primorsky honey bees removed significantly more dead brood (P=0.023) than the domestic commercial colonies tested with means of 91±2.5% (mean±SE) and 81±3.1%, respectively. The assay date (P=0.004) and apiary (P=0.009) also influenced the rate of dead brood removal. Brood removal was higher during the second assay (90±2.5%) than during the first assay (81±2.5%). Colonies in apiary two removed more (91±2.9%) dead brood than in apiary one (80±2.7%). No significant interactions were observed between bee type, time and apiary. This means that comparative brood removal of the two bee types did not vary with the assay date or apiary. The ARS Primorsky honey bees consistently removed more dead brood than the domestic colonies. Honey bee colonies that removed ≥95% in at least two assays are considered hygienic (Spivak and Downey, 1998). Following this standard, 12 ARS

[1] **USDA Honey Bee Breeding, Genetics and Physiology Laboratory, 1157 Ben Hur Road, Baton Rouge, LA 70820**

[12]**Harper's Honey Farm, 421 Louveteau Rd, Carencro, Louisiana 70520, E-mail: labeeman@net-connect.net.**

Dead brood removal	Domestic honey bees (n = 19)	Primorsky honey bees (n = 29)
High removal (≥90%)	7	20
Low removal (≤89%)	12	9
Hygienic colonies in both assays (≥95% removal)	4	12

Table. Number of domestic and ARS Primorsky honey bee colonies showing rates of dead brood removal.

Primorsky colonies (41%) consistently showed the hygienic trait as compared to only 4 of the domestic colonies (21%)(Table). More detailed examination of the results using a 90% standard shows that 20 ARS Primorsky colonies (69%) were hygienic and 7 domestic colonies (37%) were hygienic (Fisher's exact test, P=0.039).

Colony population - The domestic and ARS Primorsky honey bee colonies had similar numbers of frames of adult bees (P=0.97) and capped brood (P=0.96). The domestic colonies had 2.05±0.16 deep frames of capped brood, and 8.15 ±0.43 frames of adult bees. The ARS Primorsky honey bee colonies had 2.04±0.15 and 7.12±0.39 frames of capped brood and adult bees, respectively. Brood sizes of colonies were larger in apiary two (2.34±0.15) than in apiary one (1.75±0.16)(P=0.007). No interactions between bee type and apiary site for either brood size (P=0.062) or adult population (P=0.876) were observed. No correlation was found between rate of brood removal and the amount of adult bees present in either domestic (r= 0.085, P=0.685) or ARS Primorsky (r= -0.058, P=0.763) colonies.

Discussion

The ARS Primorsky honey bee was released to the beekeeping industry because of its ability to regulate populations of *V. destructor* in its colonies (Rinderer et al. 1999, Rinderer et al. 2000, Rinderer et al. 2001, In Press). This regulation is founded on several specific characteristics (Rinderer et al. 2001, In Press). The data presented here suggest that strong hygienic behavior is an additional characteristic of ARS Primorsky honey bees that may contribute to the general *V. destructor* resistance phenotype. This conclusion is founded on Boecking and Drescher's (1991) generalization that many hygienic colonies are able to detect pupae that are infested by *V. destructor* and then behave hygienically toward them. However, the expression of hygiene may be less intense toward *V. destructor* (Spivak 1996) than it is toward freeze-killed brood or brood killed by naturally occurring brood diseases such as American foulbrood.

Overall, ARS Primorsky honey bees hold more of the genes for hygienic behavior than the domestic European bees tested in this study. Genotype of honey bees has been long recognized to play an important role in regulating nest-cleaning behavior (Park 1936, Rothenbuhler 1964). Hence, it is reasonable to predict that future generations of purebred ARS Primorsky stock will also carry this genotype and express hygienic behavior under suitable environmental conditions.

Hygienic behavior was more strongly expressed in the second trial and in one of the two apiaries. These differences most likely resulted from differences in stored nectar or nectar flow conditions that also varied between trials and apiaries. In our first trial, the domestic colonies had fewer stores. The ARS Primorsky honey bee colonies were more frugal through the summer dearth and thus, had more stores. Also, there was no nectar flow during the first assay. Colonies of both stocks and in both yards were fed between the two trials. Then, during the second trial, a nectar flow occurred that varied in strength between the two apiary locations.

The relationships we observed in this study between both having more food while being tested and being tested in a nectar flow with enhanced hygienic behavior are congruent with previous studies. Several studies have documented the enhancement to hygienic behavior expression by both stored honey and nectar being stored ((Borchers 1964, Mourer 1964,). During strong nectar flows, "non-hygienic" colonies remove some dead brood and hygienic colonies generally remove all dead brood (Momot and Rothenbuhler 1971). This also was the case in Rothenbuhler's (1964b) classic work on hygienic behavior. During the same nectar flow conditions, the hygienic Brown line removed all disease-killed larvae during a period of about 6 days, the non-hygienic Van Scoy line removed about 80%. Thus, Rothenbuhler studied the genetics of the difference between the hygienic behavior of these two lines rather than the difference between hygienic behavior and non-hygienic behavior.

In order to overcome the difficulties for a breeding program presented by the varied expression of hygiene in varied environments, Spivak and Downey (1998), only considered colonies that removed ≥95% of dead brood in two days in at least two assays to be clearly hygienic. This conservative definition is aptly employable in a selection program where parents should express the very strongest hygiene. However, a more inclusive definition such as that of Rothenbuhler (1964) may be more useful in stock descriptions. Under either standard, the ARS Primorsky honey bees were generally hygienic. The commercial stock, although not generally hygienic by the more rigorous standard, was hygienic by a more inclusive standard.

Spivak and Gilliam (1993) observed that the expression of hygienic behavior is influenced by the strength of test colonies. In our study, no correlation between removal rate and adult population was observed. However, the colonies we studied had similar adult bee populations and detection of the correlation may require a group of colonies with greater variance.

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