Comparison of the Hygienic Behavior of ARS Russian and Commercial Honey Bees in Thailand

by BOONMEE KAVINSEKSAN[1], SIRIWAT WONGSIRI[2], THOMAS E. RINDERER[3], LILIA I. DE GUZMAN[3]

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ABSTRACT

The hygienic behavior of honey bees (*Apis* spp) is a mechanism of disease and mite resistance. Hygienic honey bees detect, uncap, and remove diseased or parasitized brood, including the parasites, from the colony. This study compared the hygienic behavior of honey bees commercially available in Thailand to that of ARS Russian honey bees, which are known for their resistance to *varroa* and tracheal mites in the United States. Ten Thai and 10 ARS Russian honey bee colonies were compared for their rates of brood removal using the liquid nitrogen technique. Results from two assays showed that both Thai and ARS Russian honey bees displayed similar ($P = 0.602$) rates of brood removal with means of 85.5 ± 3.7% and 82.6 ± 4.2%, respectively. For both stocks, 50% of the colonies were considered hygienic since they consistently showed >95% brood removal in both assays. The number of adult worker honey bees was not correlated to the rate of hygienic behavior.

INTRODUCTION

The hygienic behavior of honey bees (*Apis* spp.) is a natural defense against diseases and parasitic mites (Park 1937, Gilliam et al.1983, Boecking and Drescher 1991). Hygienic honey bees detect, uncap and remove diseased or mite-infested brood from the colonies. Hygienic behavior has been shown to help control infections of American foulbrood (Park 1937) and chalkbrood (Gilliam et al.1983), and limit the population growth of both *Varroa destructor* (Boecking et al. 1992, Spivak and Reuter 1998) and *Tropilaelaps clareae* (Ritter and Schneider-Ritter 1988, Boecking and Drescher 1990, Boecking et al. 1992).

In Thailand, *T. clareae* is a more serious pest of *A. mellifera* than *varroa* mites (Wongsiri et al. 1989b). Although chemical, cultural and combinations of chemical and cultural methods provide some control of parasitic mites (Tangkanasing et al. 1988), nothing provides complete control. In addition, these methods are either labor intensive, costly, reduce bee populations or contaminate bee products. Thus, finding honey bees with natural defenses against parasitic mites generally and especially against *T. clareae* is highly desirable (Wongsiri et al. 1989a).

The ARS Russian honey bees are known to have significant resistance to *V. destructor* and *Acarapis woodi* (Rinderer et al. 1997, 1999; de Guzman et al. 2002), and to be hygienic (de Guzman et al. 2001). The founder population of ARS Russian honey bees probably was naturally selected for mechanisms of resistance to *V. destructor* (including hygienic behavior) during the last 150 years when they were kept in the range of *V. destructor* in far-eastern Russia. Beekeepers in the area did not attempt to control the mite since, for most of the period, they were unaware of the existence of the mite, which was only discovered in 1911 (Danka et al. 1995). Although it is hygienic, (de Guzman et al. 2001) the Russian stock has never been intentionally selected for hygiene, but rather for general resistance to *V. destructor*, *A. woodi*, honey production and overwintering ability. The commercial honey bees in Thailand descended from various successful importations of honey bees (primarily but not exclusively of Italian ancestry) in the early 1970's (Wongsiri and Chen 1995). These importations led to the development of a Thai strain of *A. mellifera* which has been successfully used in commercial beekeeping. This strain may also have been selected for resistance to parasitic mites since beekeepers presumably use the best of their colonies to propagate new queens in areas plagued by *T. clareae*. This study was conducted to evaluate commercially available honey bee colonies in Thailand for hygienic behavior, which is an important character in the regulation of mite populations in the colonies.

MATERIALS AND METHODS

We evaluated the rates of brood removal of 10 Thai and 10 ARS Russian honey bee colonies in Chiang Mai, Thailand in 2002. The ARS Russian queens were obtained from the USDA-ARS, Honey Bee Breeding, Genetics and Physiology Laboratory in Baton Rouge, LA, USA while the Thai queens were provided by Supa's apiary in Chiang Mai, Thailand. All queens of both strains were introduced into standard Langstroth colonies comprised of about 9000 adult worker bees. All colonies were provisioned with two frames of honey and pollen and two empty combs. Experimental procedures were carried out about five months after queen introduction, insuring that the behavior we measured was that of the progeny of the experimental queens.

The rate of brood removal was determined using the liquid nitrogen technique as described by Spivak and Reuter (1998). A 7.5-cm diameter metal cylinder was twisted into a sealed worker brood comb of the test colonies until it reached the midrib of the comb. A volume of 150 ml of liquid nitrogen was poured into the

[1] **Department of Applied Biology, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University, Isaraphab Rd., Dhonburi, Bangkok 10600, Thailand**
[2] **Center of Excellence in Entomology: Bee Biology, Biodiversity of Insects and Mites, Department of Biology, Faculty of Science, Chulalongkorn University, Phayathai Rd., Phathumwan, Bangkok 10330, Thailand**
[3] **USDA-ARS, Honey Bee Breeding, Genetics and Physiology Laboratory, 1157 Ben Hur Road, Baton Rouge, Louisiana 70820-5502, USA**

cylinder. After the liquid nitrogen had evaporated, we poured a second 150 ml of liquid nitrogen into the cylinder to assure that the worker brood had been killed. After the comb thawed, the cylinder was removed. The number of sealed brood cells inside the brood area marked by the cylinder on each comb was then counted. The location of the test areas on each comb was marked on a clear plastic sheet to facilitate later identification of the exact area. Each comb was then returned to its colony and placed at the center of its brood nest. Two days (48 h) later, the test combs were removed from their colonies. The test areas were located with the aid of the plastic sheets and the numbers of cells which were uncapped and had no remnants of dead brood were counted. Cells that were partially uncapped, only uncapped or uncapped with traces of bee parts were not scored as having evidence of hygiene. The numbers and percentages of cells showing evidence of complete hygiene were then calculated.

The experimental procedure was conducted twice (on April 19 and May 10, 2002). The weather during the first assay was sunny and the temperature was high (36-41°C). During the second assay, it was sunny on the first day and rainy on the second day with temperatures ranging from 20-40°C. There was no nectar or pollen flow during either of the two assay periods. Each colony was fed with about 1.5 L of sugar syrup one week before each assay.

We estimated the worker population size and brood area of each colony as described by Burgett and Burikam (1985) and Rinderer et al. (1999). We then estimated total colony size as the sum of the number of adult worker bees and the number of capped brood cells for each colony.

Data on brood removal from the two assays were averaged to reduce environmental variance. Repeatability between the two assays was evaluated for each strain using a Pearson rank correlation. Differences between the hygienic behavior of the strains, numbers of adult worker bees, numbers of sealed worker brood, and total colony size were analyzed using two-tailed *t*-tests. A Pearson correlation coefficient was used to evaluate the relationship between the rate of brood removal and total colony size.

RESULTS

Our results showed that the Thai and ARS Russian stocks were similar in their rates of brood removal ($t = 0.375$, df = 18, $P = 0.712$) (Table). The two assays were quite similar: the Pearson correlation between colony scores for the Thai and ARS Russian bees were $r = 0.89$ and $r = 0.74$, respectively ($P<0.0001$ for each strain). The average brood removal of the Thai and Russian colonies for the first assay was 86.0 ± 4.7% and 86.0 ± 4.3 % ($t = 0.008$, df = 18, $P = 0.994$), and for the second assay was 85.1 ± 6.0% and 79.2 ± 7.4 ($t = 0.615$, df = 18, $P = 0.546$), respectively. According to Spivak and Reuter (1988), colonies that remove over 95% of freeze-killed brood within 48h in two assays are considered hygienic. Our results showed that 50% of the Thai and Russian colonies met this criterion.

Thai strain colonies tended to be larger than the ARS Russian colonies with moderately larger numbers of adult honey bees ($t = 1.92$, df = 18, $P = 0.07$), more capped brood ($t = 1.49$, df = 18, $P = 0.161$), and larger total colony sizes ($t = 1.98$, df = 18, $P = 0.09$) (Table). While none of these differences reaches the standard of $P = 0.05$, they all approach it. No correlation between the size of colonies and hygienic behavior scores was observed (Thai colonies: $r = 0.514$, $P = 0.13$; ARS Russian colonies: $r = 0.063$, $P = 0.863$).

DISCUSSION

This is the first time that a Thai honey bee strain has been evaluated for levels of hygienic behavior by employing a quantifiable method. The ARS Russian honey bees are known for their resistance to *V. destructor*. The regulation of *V. destructor* populations by the Russian honey bees results from the combined effects of a suite of characteristics including hygienic behavior. The percentage of hygienic colonies (50%) of the ARS Russian strain in this study was higher than the previous estimation (41%) by de

Bee strain	n	Rate of brood removal (%)	Number of adult bees	Number of sealed brood cells	Total colony size
ARS Russian	10	82.6 ± 6.8	3,847 ± 783	4,533 ± 681	8,380 ± 1,427
Thai	10	85.5 ± 5.4	6,515 ± 1,147	7,140 ± 1,601	13,656 ± 2,563

Table. Rate of brood removal, number of adult workers, number of sealed brood cells, and colony size of Thai and ARS Russian strains of *A. mellifera* (Mean ± standard error). In no case are means different at $P = 0.05$.

Guzman et al. (2001) who concluded that ARS Russian honey bees were more hygienic than a line of Italian honey bees commonly used commercially in the United States. Hence, this stock was used as a control in this study.

Our results also indicate that the Thai colonies studied were at least as hygienic as the ARS Russian strain and consequently must also be considered to be generally hygienic. This quantitative assessment of the hygienic behavior of Thai honey bees confirms and extends the observations of Ritter and Schneider-Ritter (1988) that Thai commercial honey bees uncapped brood cells infested with *Tropilaelaps* and also removed the infested brood.

Although we did not study large samples of either stock, the conditions of our evaluation of hygienic behavior further support the conclusion that both the ARS Russian and Thai honey bees are highly hygienic. Both assays were done during nectar dearth periods. Several studies have shown that hygienic behavior is more strongly expressed when nectar is abundant (Rothenbuhler 1964, Momot and Rothenbuhler 1971, Spivak and Reuter 1998). This observation further indicates a strong hygienic tendency for both strains since the common environmental effects of a lack of nectar flow did not occur in this experiment.

While the differences in colony size between the stocks were marginal, it is very important to note that the sizes of both the Thai and the ARS Russian colonies were quite small. This occurred because the experimental colonies were established when queens were available rather than during the most favorable season. Russian bees are known for being resource-oriented; the queens either slow down or completely stop brood production when food is scarce (Tubbs et al. 2003). Thus, the small population size in the ARS Russian colonies is a response to this unfavorable condition.

Spivak and Gilliam (1993) reported that the expression of hygienic behavior is generally influenced by the strength of colonies, with smaller colonies tending to be less hygienic. However, in our study, the size of colonies was not correlated with rate of brood removal. This observation is further evidence that hygienic behavior is strongly expressed by both strains. Since both strains are apparently hygienic regardless of environmental effects, it is likely that their genetics strongly predispose them to be hygienic.

Certainly hygienic behavior of honey bees is regulated by genes (Rothenbuhler 1964). Furthermore, most strains of *A. mellifera* are not strongly hygienic and selection enhances the trait (Spivak and Reuter 1998, 2001). Colonies of both strains we studied have high levels of hygienic behavior. However, neither of them have been selected specifically for hygienic behavior. We suggest that this resistance may have arisen as a result of being maintained and propagated in areas with parasitic mites, a condition which might produce selection for hygienic behavior. This character may have resulted from Thai beekeepers propagating from their strongest colonies in areas infested by *T. clareae*. Beekeepers may have inadvertently selected for strong hygienic behavior and perhaps other mechanisms of resistance to parasitic mites in both the Thai and Russian stocks. Using the same Thai and Russian colonies, we also observed that about 50% of brood cells infested with *T. clareae* contained non-reproductive females (Kavinseksan 2003).

Most importantly, this study indicates that a Thai strain of *A. mellifera* possess a hygienic trait, which may be helpful in regulating *T. clareae* populations in the colonies. This further indicates that a selection program with honey bees specifically directed toward increasing resistance to *T. clareae* may produce a stock with sufficient resistance to slow the growth of *T. clareae* populations, which might reduce levels of acaricide use. Thus, if *Tropilaelaps* mites should be introduced to areas outside their present range, such as the United States, selection of honey bees for genetic resistance should be successful.

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