CHAPTER 6

COMPARISON OF THE HYGIENIC BEHAVIOR OF THAI COMMERCIAL AND ARS RUSSIAN HONEY BEES

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 *Apis mellifera* commercially available in Thailand to that of ARS Russian (or Primorsky) 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% (Mean± standard error) 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.

Key words: Hygienic behavior / Russian bees / *A. mellifera* / Thailand

Introduction

The hygienic behavior of honey bees (*Apis* spp.) is a natural defense against diseases and parasitic mites (Park, 1937; Gilliam and Taber, 1983; Boecking and Drescher, 1991). Hygienic honey bees detect, uncap and remove diseased or mite-infested brood from the colonies. In *A. mellifera*, hygienic behavior has been shown to help control infections of American foulbrood (Park, 1937) and chalkbrood (Gilliam and Taber, 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). Likewise, hygienic behavior is an important mechanism for resistance to *V. jacobsoni* by *Apis cerana* (Wongsiri et al., 1989a; Rath, 1991) and to *T. clareae* by *Apis dorsata* (Burgett and Rossignol, 1990).

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 *A. mellifera* with natural defenses against parasitic mites generally and especially in the Asian context against *T. clareae* is highly desirable (Wongsiri et al., 1989a).

The ARS Russian honey bees (*A. mellifera*) are known to have significant resistance to *V. destructor* and *Acarapis woodi* (Rinderer et al., 1997, 1999), 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). The commercial *A. mellifera* 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 *A. mellifera* colonies in Thailand for hygienic behavior, which is an important character in the regulation of mite populations in the colonies.

Materials and Methods

The rates of brood removal were evaluated from 10 Thai (or domestic) and 10 ARS Russian (or Primorsky) 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, Louisiana, 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 cylinder. After the liquid nitrogen had evaporated, a second 150 ml of liquid nitrogen was poured 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 ranged 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.

The worker population size and brood area of each colony were estimated as described by Burgett and Burikam (1985) and Rinderer et al. (1999). Total colony size were then estimated 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

The results in this study showed that Thai and ARS Russian stocks were similar in their rates of brood removal ($t = 0.375$, $df = 18$, $P = 0.712$) (Table 6.1). 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 $86.0 \pm 4.7\%$ and 86.0 ± 4.3 % ($t = 0.008$, $df = 18$, $P = 0.994$), and for the second assay was $85.1 \pm 6.0\%$ and 79.2 ± 7.4 ($t = 0.615$, $df = 18$, $P = 0.546$), respectively. According to Spivak and Reuter (1998), colonies that remove over 95% of freeze-killed brood within 48h in two assays are considered hygienic. Following this standard, 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 6.1). While none of these differences reaches the standard of *P* = 0.05, they all approach it.

Table 6.1 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.

Bee strain	n	Brood removal rate (%)	Number of adult bees	Number of sealed brood cells	Total colony size	Percentage of hygienic colonies
ARS Russian	10	82.6 ± 6.8	3,847 ± 783	4,533 ± 681	8,380 ± 1,427	50
Thai	10	85.5 ± 5.4	6,515 ± 1, 147	7,140 ± 1,601	13,656 ± 2,563	50

Discussion

This is the first time that the Thai *A. mellifera* 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*, and are used commercially in the United State (Rinderer et al., 1999, 2000; Spivak and Reuter, 2001). The regulation of *V. destructor* populations by the Russian honey bees results from the combined effects of a suite of characteristics including hygienic behavior (De Guzman et al., 2001). Hence, this stock was used as a standard in this study.

The percentage of hygienic colonies (50%) of the ARS Russian strain in this study was higher than the previous estimation (41%) by de Guzman et al. (2001) who concluded that ARS Russian honey bees were hygienic as compared to a line of Italian honey bees commonly used commercially in the United States. The 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.

The conditions of this 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 dearths. 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.

The differences in colony size between the Thai and the ARS Russian colonies were marginal. It is very important to note that the sizes of the both stocks 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 this study, the size of colonies was not correlated with hygienic behavior scores (Thai colonies: $r = 0.514$, $P = 0.13$; ARS Russian colonies: $r = 0.063$, $P = 0.863$). 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 artificial selection is required to enhance the trait (Spivak and Reuter, 1998, 2001). Both strains in this study have high levels of hygienic behavior and this resistance has arisen as a result of being maintained and propagated in areas with parasitic mites, although neither strain has been specifically selected 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 *T. clareae* such as grooming behavior and non-reproduction of mites.

Most importantly, this study indicates that the Thai strain of *A. mellifera* possess a hygienic trait, which may be helpful in regulating *T. clareae* population in the colonies. A selection program with the commercial Thai honey bee strain specifically directed toward increasing its resistance to *T. clareae* may produce a stock with sufficient resistance to slow the growth of *T. clareae* populations, which would reduce levels of acaricide use.

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