Journal of Apicultural Research 44(1): 21–23 (2005)

ORIGINAL ARTICLE

Suppressed mite reproduction explained by the behaviour of adult bees

JOHN R HARBO* AND JEFFREY W HARRIS

USDA/ARS Honey Bee Breeding, Genetics and Physiology Lab, Baton Rouge, Louisiana 70820 USA

Received 1 November 2004, accepted subject to revision 24 January 2005, accepted for publication 4 February 2005

SUMMARY

Suppressed mite reproduction (SMR) is a heritable trait of the honey bee (*Apis mellifera*) that can control the parasitic mite, *Varroa destructor*. The purpose of this study was to determine whether adult bees with the SMR trait affect mites in brood after cells are capped. Colonies with or without the SMR trait were each given a comb of newly-capped worker brood that was naturally infested with varroa. Each of 7 source colonies provided a comb of brood to at least one SMR (n = 9) and one control colony (n = 8). These combs were removed from their host colonies 8 days later and mite populations evaluated in cells with bee pupae that were >8 days post-capping. Colonies with SMR bees averaged 2.2% of their cells infested with mites; controls averaged 9.0%. Therefore, bees with the SMR trait apparently removed mites from capped cells. Of the mites that remained, the SMR colonies had a much lower rate of reproductive mites, 20% vs. 71%. This suggests that bees with the SMR trait removed reproductive mites more often than they removed non-reproductive mites. When comparing only the number of mites that produced no progeny, the groups were almost identical averaging 1.2 and 1.3 mites per 100 cells of brood. This suggests that the SMR bees did not remove mites from brood cells if the mites did not lay eggs. By targeting the reproductive mites, bees with the SMR trait give the illusion that nearly all of the mites are non-reproductive. Therefore, our selection for a low frequency of reproductive mites may have produced bees that remove reproductive mites from capped brood.

Keywords: *Apis mellifera*, *Varroa destructor*, SMR, hygienic behaviour, parasitic mites, honey bees, resistance

INTRODUCTION

When mite-resistant bees were detected in various parts of the world, the colonies often had a high proportion of non-reproducing mites in their brood cells (Ruttner *et al.*, 1984; Ritter, 1990; Eguaras *et al.*, 1995). Fuchs (1994) found that this non-reproductive trend was only slightly affected by the ability of bee brood to inhibit the reproduction of mites, but until now we could not explain how certain bee colonies could cause a high proportion of their mites to be non-reproductive.

After learning that the frequency of non-reproducing mites in brood could be affected by a heritable component in bees (Harbo & Harris, 1999), we began selecting colonies for a low frequency of reproducing mites. Our definition of non-reproductive mites combined three categories: (1) a dead foundress with no progeny, (2) a live foundress that laid no eggs, and (3) a foundress with nonviable offspring. Although we agree with Correa-Marques *et al.* (2003) that males are necessary to produce viable females, our working definition of nonviable offspring did not include one or more viable females without a male. If a cell could produce at least one adult female mite, we recorded it as reproductive. In our breeding work, we measured mite reproduction in capped worker brood and used this as a basis for selection. To calculate percent reproductive mites in a colony, we examined about 20 singly infested cells when the host pupae were 8–11 days post-capping (purple-eyed pupae and older). With this criterion, the use of single-drone inseminations, and about 5 generations of selection, we produced colonies that had < 6% of their mites classified as reproductive in worker cells. We found that varroa mites could be controlled with this single mite-resistance trait (Harbo & Harris, 2001), and we used the term suppressed mite reproduction (SMR) when describing this trait of bees that produced colonies that had a low frequency of reproductive mites in worker brood.

Ibrahim & Spivak (2004) found that the bees that were selected as described above (colonies with the SMR trait) were very hygienic and were able to remove varroa mites from capped cells. Since some honey bees have demonstrated an ability to detect and remove varroa from infested cells (Boecking & Drescher, 1991, 1992; Spivak, 1996; Correa-Marques & De Jong, 1998; Nazzi *et al.*, 2004), Ibrahim & Spivak (2004) hypothesized that removal of brood infested with reproductive mites could explain part or all of what we describe as the SMR trait. However, Ibrahim & Spivak (personal communication) concluded that colonies of bees with the SMR trait control mites through a brood effect as well as through the behaviour of adult bees.

We designed this study to determine whether adult bees with the SMR trait would affect mite populations after the mites had entered a brood cell. We transferred combs of capped worker brood that were naturally infested with varroa from source colonies into SMR and control colonies. Our results suggest that the SMR trait may be identical to varroa-specific hygiene described by Boecking *et al.* (2000). Clearly, suppressed mite reproduction may no longer be the best name for this trait, but for consistency, we will use the name SMR throughout this paper.

MATERIALS AND METHODS

At least two frames of newly capped worker brood were removed from each of seven source colonies that were infested with varroa. These source colonies had moderate mite populations (5–10% of the brood cells infested) and more than 65% of the mites were reproductive. This rate of infestation was not so high as to produce obvious pathogenic effects in the colony but high enough to make it relatively easy for us to find mites for evaluation.

On 11 August, 2–4 combs were randomly transferred from each source colony to at least one SMR (n = 9) and one control colony that did not express the SMR trait (n = 8). Recipient colonies had been evaluated about two weeks earlier for percent reproductive mites, and these measurements (as well as the pedigree of the SMR bees) were the bases for creating the two groups. SMR colonies averaged 3 ± 6% reproductive mites (mean ± s.d.); control colonies averaged 80 ± 10%. A frame of brood was removed from the recipient colonies when the source comb was introduced. This allowed the brood nest in each colony to

*Corresponding author: jharbo@ars.usda.gov

retain its size. Combs remained in the recipient colonies for 7–9 days.

At the end of the 7–9 day period (18–20 August), we examined the combs for mite reproduction. Using the age of the bee pupa as a guide, we examined only worker cells that were aged 8–11 days post-capping. For combs that we examined on the morning of 20 August (when combs had been in recipient colonies for almost 9 days), we examined only cells that were at least nine days post-capping. Combs from the same source colony were all removed from their recipient colonies at the same time.

We compared SMR colonies with control colonies for (1) the rate of cell infestation (the combs were assumed to be equal when removed from the same source colony), (2) the proportion of the infested cells that had reproductive mites (a mite that had at least one daughter that was old enough to mature before the host bee emerged), and (3) the number of cells with mites that laid no eggs. We examined 300–800 cells with tan-bodied pupae in each colony. If we found fewer than 18 infested per 300 cells, we checked more cells until we had examined 800 cells or all of the brood cells of the correct age. In 8 control colonies, we found 18–44 infested cells (mean = 28); in 9 SMR colonies, we found 5 - 20 (mean = 12). Cells of worker brood with mites were counted as reproductive (at the tan pupa stage) if they contained at least one female mite that was older than a protonymph.

Data were analysed with SAS (2000) software (version 8) using a complete randomized block design. The seven source colonies served as random block effects; treatment (SMR or control) served as a fixed effect (Proc Mixed). Three of the source by treatment combinations were replicated twice, and the subsampling variance for those source colonies was included in the model. The Kenward-Roger estimate for degrees of freedom was used to pool the subsampling variance with the source by treatment variance when it was appropriate to do so.

RESULTS

After the newly capped cells had spent 7–9 days in the test colonies, colonies with SMR bees averaged 2.2 infested cells per hundred cells examined; controls averaged 9.0. Of the infested cells, 20% were reproductive in the SMR colonies, whereas 71% were reproductive in control colonies. Both results were statistically different (table 1). The two groups were not different in the number of mites that produced no eggs (table 1).

DISCUSSION

The results suggest that adult bees with the SMR trait affect mite populations after the mites have entered brood cells. We did not watch the behaviour of adult bees or monitor the fate of infested brood cells, but selective removal of mites from capped cells seems to us to be the most plausible explanation.

The data also suggest that SMR bees removed reproductive mites more often than non-reproductive mites. If mites were removed at random, the frequency of reproductive mites would be equal in the two groups. This was clearly not the case (table 1). Moreover, the two groups ended the test with equal numbers of mites that produced no eggs (table 1). This suggests that SMR bees may locate infested cells by detecting something associated with the reproductive activities of mites.

Our colonies with SMR bees apparently removed most of the reproductive mites from brood cells, thereby leaving a high proportion of non-reproductive mites in brood. Therefore, when we examined older brood from colonies with SMR bees we found few mites, most of which were not reproductive. We concluded that the mite populations in those colonies had become non-reproductive. In a sense they did, but not in the way that we thought. Although we do not know many of the details surrounding the SMR trait (such as the fate of mites after removal) the removal of reproductive mites is a concise mechanism of resistance that can be traced to a specific behaviour by the bee.

We found no evidence that bees responded to adult mites in brood cells (as long as the mites did not lay eggs). Boecking & Drescher (1992) and Spivak (1996) reported that bees were more likely to be hygienic when cells were artificially infested with two rather than one mite, and their findings may indicate that bees were responding to adult mites. However, the presence of more adult mites may have been an indirect stimulus for removal. In their studies, bees may have removed the contents of doubly infested cells at a higher frequency than singly infested cells because doubly infested cells are more likely to have mite progeny, even if from only one of the foundresses. Spivak (1996) reported a high level of non-reproduction among mites that had been artificially introduced into cells, so if 40% of the artificially introduced mites produced no progeny then one would expect only 16% of the doubly infested cells to have no progeny. Therefore, as with our results, their results could be explained by worker bees targeting cells with reproductive mites.

The removal of reproductive varroa by adult bees unifies the reports of varroa hygiene (Boecking & Drescher, 1991, 1992; Spivak, 1996; Correa-Marques & De Jong, 1998; Boecking *et al.*, 2000; Nazzi *et al.*, 2004) with those that relate mite resistance to a high frequency of non-reproductive mites (Ruttner *et al.* 1984; Ritter, 1990; Eguaras *et al.*, 1995; Harris & Harbo, 2000). All are probably linked to varroa hygiene.

Acknowledgements

Daniel Winfrey and David Dodge (ARS-USDA, Baton Rouge) provided technical support. Deborah Boykin (ARS-USDA, Stoneville, MS) provided statistical guidance. The work was done in cooperation with the Louisiana Agricultural Experiment Station.

REFERENCES

BOECKING, O; DRESCHER, W (1991) Response of *Apis mellifera* L colonies to brood infested with *Varroa jacobsoni* Oud. *Apidologie* 22: 237–241.

BOECKING, O; DRESCHER, W (1992) The removal response of *Apis mellifera* L colonies to brood in wax and plastic cells after artificial and natural infestation with *Varroa jacobsoni* Oud. and to freeze-killed brood. *Experimental and Applied Acarology* 16: 321–329.

BOECKING, O; BIENEFELD, K; DRESCHER, W (2000) Heritability of the Varroa-specific hygienic behaviour in honey bees (Hymenoptera: Apidae) *Journal of Animal Breeding and Genetics* 117(6): 417–424.

TABLE 1. Analyses of mite populations after newly capped cells of naturally infested brood were transferred into suppressed mite reproduction (SMR) or control colonies. Mite populations were evaluated 8 ± 1 days later by examining cells about 9 days post-capping.

Variable	Least significant means ± s.e. SMR bees[a]	Control bees[a]	F	df	P
Percentage of cells that were infested with varroa	2.2 ± 0.8%	9.0 ± 0.9%	30.5	1,15	0.0001
Frequency of mite reproduction among infested cells	20 ± 5%	71 ± 5%	60.5	1,8	0.0001
Percentage of cells with non-laying mites	1.2 ± 0.2%	1.3 ± 0.2%	0.16	1,15	0.70

[a]Because of lower mite populations, we examined more cells in SMR than in control colonies in order to achieve comparable accuracy in both treatment groups. We examined 563 ± 180 (mean ± s.d.) cells in each of 9 SMR colonies and 315 ± 35 cells in each of 8 control colonies.

CORREA-MARQUES, M H; DE JONG, D (1998) Uncapping of worker bee brood, a component of the hygienic behavior of Africanized honey bees against the mite *Varroa jacobsoni* Oudemans. *Apidologie* 29:283–289.

CORREA-MARQUES, M H; MEDINA, L M; MARTIN, S J; DE JONG, D (2003) Comparing data on the reproduction of *Varroa destructor*. *Genetics and Molecular Research* 2: 1–6.

EGUARAS, M; MARCANGELI, J; OPPEDISANO, M; FERNANDEZ, N (1995) Mortality and reproduction of *Varroa jacobsoni* in resistant colonies of honey bees (*Apis mellifera*) in Argentina. *Bee Science* 3: 174–178.

FUCHS, S (1994) Nonreproducing *Varroa jacobsoni* Oud. in honey bee worker cells – status of mites or effects of brood cells? *Experimental and Applied Acarology* 18: 309–317.

HARBO, J R; HARRIS, J W (1999) Heritability in honey bees (Hymenoptera: Apidae) of characteristics associated with resistance to *Varroa jacobsoni* (Mesostigmata: Varroidae). *Journal of Economic Entomology* 92: 261–265.

HARBO, J R; HARRIS, J W (2001) Resistance to *Varroa destructor* (Mesostigmata: Varroidae) when mite-resistant queen honey bees (Hymenoptera: Apidae) were free-mated with unselected drones. *Journal of Economic Entomology* 94:1319–1323.

HARRIS, J W; HARBO, J R (2000) Changes in reproduction of *Varroa destructor* after honey bee queens were exchanged between resistant and susceptible colonies. *Apidologie* 31: 689–699.

IBRAHIM, A; SPIVAK, M (2004) The relationship between suppression of mite reproduction (SMR) and hygienic behavior. *American Bee Journal* 144 (5): 406.

NAZZI, F.; DELLA VEDOVA, G; D'AGARO, M (2004) A semiochemical from brood cells infested by *Varroa destructor* triggers hygienic behaviour in *Apis mellifera*. *Apidologie* 35: 65–70.

RITTER, W (1990) Development of varroa mite population in treated and untreated colonies in Tunisia. *Apidologie* 21: 368–370.

RUTTNER, F; MARX, H; MARX, G (1984) Beobachtungen über eine mögliche Anpassung von *Varroa jacobsoni* an *Apis mellifera* L. in Uruguay. *Apidologie* 15: 43–62.

SAS INSTITUTE (2000) *OnlineDoc, version 8*. SAS Institute Inc.; Cary, NC, USA.

SPIVAK, M. (1996) Honey bee hygienic behavior and defense against *Varroa jacobsoni*. *Apidologie* 27: 245–260.