Attractiveness of Africanized Honey Bee Brood From Southern Texas to *Varroa destructor* Infestation

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Abstract

The attractiveness to *Varroa destructor* infestation of Africanized honey bee (AHB) brood from southern Texas and European honey bees (EHB) from Louisiana were compared using a bioassay. The overall conclusion is that the larvae of AHB in South Texas and EHB from Louisiana were equally attractive to *V. destructor* and that the mites reproduced as successfully. Small differences were detected which suggest that AHB may have a slight resistance to *V. destructor*. However, other aspects of the epizootiology of varroosis such as climate, variation in honey bee stock, and variation in *V. destructor* determine the severity of the parasitism substantially more than the small, generally insignificant differences reported here. Possible implications of these observations on feral populations of AHB are discussed.

KEY WORDS

Africanized honey bees, European honey bees, *Varroa destructor*, attractiveness, resistance, Texas

INTRODUCTION

There are two major biological problems confronting the U. S. beekeeping industry. One is the challenge of parasitic mites and the other is Africanized honey bees (AHB) [*Apis mellifera scutellata* Lepeltier hybridized with European honey bees (EHB)] (Gary 1991). *Varroa destructor* (Anderson and Trueman, 2000) is an ectoparasitic mite causing a threat to beekeeping with *Apis mellifera* L. The AHB is a concern primarily because of its negative public relations impact on the beekeeping industry. In the U. S., AHB is well established in southern Texas, Arizona, southern New Mexico, and southern California, and its distribution is expected to slowly expand.

Several studies with African or Africanized honey bees in Brazil and Mexico have shown that they are more resistant or attractive to *V. destructor* infestation than EHB (Moritz and Hanel 1984, Ruttner *et al.* 1984, Camazine 1986, Moritz and Mautz 1990, Moretto *et al.* 1991a & b, Medina and Martin 1999). The described resistance mechanisms are reduced attraction by brood, shorter postcapping duration, higher proportion of non-reproductive females, smaller sized brood cells, and more efficient grooming behavior. However, climate also was postulated to influence the reproductive success of this parasite (de Jong *et al.* 1984, Moretto *et al.* 1991a).

The reproductive ability and virulence of *V. destructor* to AHB colonies in the U. S. have not been studied. This study was undertaken to determine the attractiveness of AHB brood from southern Texas to *V. destructor* infestation compared to European-derived brood from Louisiana. The reproductive ability of *V. destructor* in both bee types was also examined.

MATERIALS AND METHODS

This study was conducted in Weslaco, Texas, in February 1995. Larval attractiveness and mite reproduction to *V. destructor* infestation of AHB was compared to that of EHB using a bioassay. This bioassay provided larvae of both bee types with similar chances of becoming infested in a common environment and was achieved by grafting or manual transfer of larvae of both types into empty cells of the same comb and placing the comb into an infested colony (de Guzman *et al.* 1995).

Ten AHB test colonies with a probability of Africanization ranged from 0.964 to 1.000 (Rinderer *et al.* 1993) were selected from two apiaries located near Rio Grande City, Texas, maintained by the USDA, ARS, SARL, Honey Bee Research Unit, Weslaco, Texas. Selection was based on their high probability of Africanization. Measurements of restriction fragment length polymorphisms revealed that one of the ten AHB test colonies had EHB mitochondrial DNA. This colony was included in the analysis since official USDA tests indicated them to be Africanized, without culling out various degrees and kinds of hybrids.

In order to assure that the EHB colonies were not marginally Africanized, ten EHB test colonies were randomly selected from colonies maintained by the USDA, ARS, Honey-Bee Breeding, Genetics and Physiology Research Laboratory apiaries in Baton Rouge, Louisiana. Three EHB inoculation colonies were also identified in Louisiana. These colonies had worker brood having *V. destructor* infestations of 19, 24 and 26%. The colonies were transported to Weslaco for the test. AHB has not been detected in Louisiana.

The grafting technique described by de Guzman *et al.* (1995) was employed. Young larvae of the 10 AHB and 10 EHB test colonies were grafted into sections (20 cells x 20 rows) of two brood combs taken from an inoculation colony. Each row contained larvae from only one test colony. Larvae from the two bee types were grafted into alternating rows, with a total of ten pairs per brood comb. The two brood combs were then placed back into the inoculation colony where the combs originated, and which was naturally infested with *V. destructor*.

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Infestation parameter	European honey bees (n = 10 colonies)	Africanized honey bees (n = 10 colonies)
Varroa prevalence	39. 47 ± 2.80	31.25 ± 2.80
Average foundress intensity	1.39 ± 0.06	1.22 ± 0.06
Foundress reproduction	2.26 ± 0.08	2.29 ± 0.08
% infested cells with non-reproductive females	5.92 ± 1.74	10.56 ± 1.75
Varroa abundance	2.04 ± 0.20	1.37 ± 0.20
Varroa mean intensity	4.53 ± 0.13	3.99 ± 0.13

Table 1. Comparative attractiveness of European (Louisiana) and Africanized (Texas) honey bee brood to *Varroa destructor* (least square means ± standard error). Total number of brood cells examined = 768 (AHB), 875 (EHB). Number of infested cells analyzed = 245 (AHB), 358 (EHB).

All pupae were examined for mite infestations on the eleventh day after grafting.Several variables were measured: *V. destructor* prevalences (proportion of potentially infested cells that were infested), average foundress intensity (number of founding females per infested cell), foundress reproduction (number of progeny per foundress including males and all stages of progeny), non-reproductive infestation rate (proportions of infested cells containing non-reproductive females), abundance (number of mites per cell ; all mites of both sexes and all stages of development divided by the total number of cells examined) and intensity (number of mites per infested cell; all mites of both sexes and all stages of development divided by the number of infested cells).

Data were analyzed using analysis of variance. The effects in the model included inoculation colony (df = 2), comb within inoculation colony (df = 3), blocks within comb x inoculation colony combination (df = 54), bee type (df = 1), and bee type within comb x inoculation colony combination (df = 5). To compensate for the lack of randomization in assigning bee types to rows within a frame, a more conservative *F*-test was used to test for bee type differences. The error term used to test bee type differences was the bee type within comb x inoculation colony combination.

RESULTS

When young larvae of AHB and EHB were simultaneously grafted into a brood comb and exposed to *V. destructor,* they had similar attractiveness to infestation (Tables 1 and 2). AHB and EHB showed similar *V. destructor* prevalences (proportion of cells infested) (E>A, $P = 0.09$), average foundress intensity (number of founding females per infested cell) (E>A, $P = 0.11$), foundress reproduction (number of progeny per female) (A>E, $P = 0.78$), proportions of infested cells containing non-reproductive females (A>E, $P = 0.12$), and abundance (number of mites per experimental cell) (E>A, $P = 0.06$). However, the trends of differences within this group of variables, are consistent (with the exception of average foundress reproduction) in suggesting that AHB colonies may have a slight degree of resistance to *V. destructor* infestation. Small numerical differences in prevalence, average foundress intensity and non-reproductive rate combined in the calculation of *V. destructor* abundance and produced a nearly significant (P= 0.06) greater mite abundance (number of *V. destructor* per cell) for EHB larvae and a clearly significant (P= 0.03) greater mean intensity (number of *V. destructor* per infested cell) for EHB larvae.

DISCUSSION

The overall conclusion from the data presented here is that worker larvae of AHB in South Texas were as attractive to North American *V. destructor* as larvae of the EHB. Varroa mites reproduced equally well on worker brood of both bee types, which corroborates the findings of Guzman-Novoa *et al.* (1996, 1999) in Mexico. However, different mite reproduction rates were attained when EHB colonies in England, examined about 4 years earlier, were compared to that of AHB colonies located in Mexico (Medina and Martin 1999). In Brazil, the reproductive success of varroa also differed significantly between the two bee types (Ritter and de Jong 1984, Camazine 1986). The small differences in reproductive success detected by this study do not support the conclusion that AHB may have a slight resistance advantage. Other aspects of the epizootiology of varroosis determine the severity of the parasitism substantially more than do the small, generally insignificant differences reported here.

Climate is reported to have a strong effect on the population growth of *V. destructor* (de Jong *et al.* 1984, Moretto et al. 1991a). In Brazil, higher levels of infestation were observed in the cooler (Sao Joaquin) than hotter (Ribeirao Preto) regions for AHB. Thus, differences in rates of varroa infestation may have been caused by climatic factors.

Honey bee stock has profound effects on the population dynamics of *V. destructor*. AHB is generally thought to be more resistant to *V. destructor* in Brazil. Reduced reproductive rates for *V. destructor* infesting AHB were observed in Brazil (Camazine, 1986) and in Mexico (Medina and Martin, 1999). Rozenkranz *et al.* (1988) observed about 51% of infesting female mites were non-reproductive in Brazilian AHB colonies and 17% in *A. m. carnica* colonies. Interestingly, both these observations are higher than our observations of about 10% for AHB in south Texas. Recent hybridization or natural selection may have produced a different AHB biotype in Texas that has far less resistance to *V. destructor* than the AHB found in Brazil.

Biotypes of *V. destructor* may have vastly different interactions with honey bees. Kraus and Hunt (1995) have shown different types of *V. jacobsoni* using random amplification of polymorphic DNA (RAPD). Using the same technique, de Guzman *et al.* (1997, 1998, 1999) documented that *V. destructor* collected from Louisiana were genetically different from *V. destructor* obtained from Brazil and determined that the mites in Louisiana originated from Russia via Europe and the mites in Brazil originated from Japan. The occurrence of these two biotypes of *V. destructor* was confirmed by Anderson and Trueman (2000) in their revision of the taxonomy of the genus that resulted in the establishment of the

	Type mean square	Error mean square	F value	*P*
Varroa prevalence	0.20	0.05	4.30	0.09
Average foundress intensity	0.84	0.23	3.69	0.11
Foundress reproduction	0.03	0.03	0.09	0.78
% infested cells with non reproductive female	0.06	0.02	3.53	0.12
Varroa abundance	13.29	2.39	5.56	0.06
Varroa mean intensity	8.64	1.06	8.15	0.03

Table 2. Results of the analysis of variance of the comparative attractiveness of European honey bee brood from Louisiana and Africanized honey bees from southern Texas.

nomen "*destructor*". Additionally, the occurrence of the "Japanese biotype" in Japan, Brazil and Puerto Rico coincides with a reduced virulence on both AHB and EHB (de Guzman and Rinderer, 1998, 1999) and EHB in Brazil (de Jong and Soares, 1977). In Argentina, which has the "Russian biotype" of varroa similar to the U. S. and Europe (de Guzman and Rinderer 1999), honey bee colonies need chemical treatment in order to survive (Rosenkranz 1999). Therefore, it is probable that the biotype of *V. destructor* used in several studies conducted in South America were different from the biotype of *V. destructor* used in our study. The greater apparent attractiveness of the AHB in Texas may actually be a greater reproductive ability and virulence by the *V. destructor* found in Texas. Definitive experiments with both biotypes of honey bees and both biotypes of *V. destructor* must wait until all four biotypes are found to occur in the same locale.

It is apparent that the suggestions of comparative "resistance" to *V. destructor* found in our study for AHB are not nearly as strong as the evidence for comparative "resistance" found for AHB in Brazil. Similarly, Mexican honey bees are also less tolerant to varroa than Brazilian honey bees (Guzman-Novoa *et al.* 1999). U. S. and Mexico have the same varroa genotype, which is different to that of Brazil (de Guzman and Rinderer 1999). Regardless of whether climate, specific honey bee biotype, specific *V. destructor* biotype, or some interaction among these three is the cause of this difference, the AHB in Texas are susceptible to *V. destructor* in Texas for the parameters of the study. It may be that some other parameter not yet studied for North American AHB will provide North American AHB with sufficient resistance to allow survival regardless of *V. destructor*. However, until such resistance is found, it is reasonable to expect that *V. destructor* will cause mortality among feral populations of AHB in the U.S. and become an important factor in influencing AHB range and abundance.

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REFERENCES

Anderson, D. L. and Trueman, J. W. H. 2000. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Exp. App. Acarol.* 24:165-189.

Camazine, S. 1986. Differential reproduction of the mite, *Varroa jacobsoni* (Mesostigmata: Varroidae), on Africanized and European honey bees (Hymenoptera: Apidae). *Ann. Entomol. Soc. Am.* 79:801-803.

de Guzman, L. I. and Rinderer, T. E. 1998. Distribution of the Japanese and Russian genotypes of *Varroa jacobsoni. Honey Bee Science* 19:115-119.

de Guzman, L. I. and Rinderer, T. E. 1999. Identification and comparison of *Varroa* species infesting honey bees. *Apidologie* 30:85-95.

de Guzman, L. I., Rinderer, T. E. and Lancaster, V. A. 1995. A short test evaluating larval attractiveness of honey bees to *Varroa jacobsoni. J. Apic. Res.* 34:89-92.

de Guzman, L. I., Rinderer, T. E. and Stelzer, J. A. 1997. DNA evidence of the origin of *Varroa jacobsoni* in the Americas. *Biochemical Genetics* 35:327-335.

de Guzman, L. I., Rinderer, T. E., Stelzer, J. A. and Anderson, D. 1998. Congruence of RAPD and mitochondrial DNA markers in assessing *Varroa jacobsoni* genotypes. *J. Apic. Res.* 37:49-51.

de Guzman, L. I., Rinderer, T. E. and Stelzer J. A. 1999. Occurrence of two genotypes of *Varroa jacobsoni* Oud. in North America . *Apidologie* 30:31-36.

de Jong, D. and Soares, A. E. E. 1997. An isolated population of Italian bees that survived *Varroa jacobsoni* infestation without treatment for over 12 years. *Am. Bee J.* 137:742-745.

de Jong, D., Gonçalves, L. S. and Morse, R. A. 1984. Dependence on climate of the virulence of *Varroa jacobsoni. Bee World* 65:117-121.

Gary, N. E. 1991. *Varroa* mites, tracheal mites, and Africanized bees - The challenges ahead. *Am. Bee J.* 131:34-39.

Guzman-Novoa, E., Sanchez, A., Page, R. E. and Garcia, T. 1996. Susceptibility of European and Africanized honey bees (*Apis mellifera* L) and their hybrids to *Varroa jacobsoni* Oud. *Apidologie* 27:93-103.

Guzman-Novoa, E., Vandame, R. and Arechavaleta, M. E. 1999. Susceptibility of European and Africanized honey bees (*Apis mellifera* L) to *Varroa jacobsoni* Oud. in Mexico. *Apidologie* 30:173-182.

Kraus, B. and Hunt, G. 1995. Differentiation of *Varroa jacobsoni* Oud. populations by random amplification of polymorphic DNA (RAPD). *Apidologie* 26:283-290.

Medina, L. M. and Martin, S. J. 1999. A comparative study of *Varroa jacobsoni* reproduction in worker cells of honey bees (*Apis mellifera*) in England and Africanized bees in Yucatan, Mexico. *Exp. Appl. Acarol.* 23:659-667.

Moretto,G., Gonçalves, L. S., de Jong, D. and Bichuette, M. Z. 1991a. The effects of climate and bee race on *Varroa jacobsoni* Oud. infestations in Brazil. *Apidologie* 22:197-203.

Moretto, G., Gonçalves, L. S. and de Jong, D. 1991b. Africanized bees are more efficient at removing *Varroa jacobsoni.* Preliminary data. *Am. Bee J.* 131. 434.

Moritz, R. F. A. and Hanel, H. 1984. Restricted development of the parasitic mite *Varroa jacobsoni* Oud. in the cape honey bee *Apis mellifera capensis. Esch. Z. Angew. Ent.* 97:91-95.

Moritz, R. F. and Mautz, D. 1990. Development of *Varroa jacobsoni* in colonies of *Apis mellifera capensis* and *Apis mellifera carnica. Apidologie* 21:53-58.

Rinderer, T. E., Buco, S. M., Rubink, W. L., Daly, H. V., Stelzer, J. A., Riggio, R. M. and Baptista, F. C. 1993. Morphometric identification of Africanized and European honey bees using large reference populations. *Apidologie* 24:569-585.

Ritter, W. and de Jong, D. 1984. Reproduction of *Varroa jacobsoni* in Europe, the Middle East, and tropical South America. *Z. Angew. Entomol.* 98: 55-57.

Rosenkranz, P., Issa, M., Rachinsky, A., Strambi, A. and Strambi, C. 1988. Honeybee-*Varroa* relationships: a comparison of Africanized and Carniolan colonies. In: *Present Status of Varroatosis in Europe and Progress in the Varroa Control.* Ed. Cavalloro: Udine, Italy pp. 193-198.

Rosenkranz, P. 1999. Honey bee (*Apis mellifera* L.) tolerance to *Varroa jacobsoni* Oud. in South America. *Apidologie* 30:159-172.

Ruttner, F., Marx, H. and Marx, G. 1984. Observations on the possible adaptation of *Varroa jacobsoni* to *Apis mellifera* in Uruguay. *Apidologie* 15:43-62.