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Original article

Parasitism in the social bee *Apis mellifera*: quantifying costs and benefits of behavioral resistance to *Varroa destructor* mites

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Abstract – The case of a host-parasite relationship may provide a good model to evaluate the costs and benefits of some behaviors, an area in which field data a currently lacking. European (EHB) and Africanized (AHB) honey bees are two *Apis mellifera* subspecies that coexist in Mexico, the former highly compatible with *Varroa destructor*, the latter less compatible. Here we examine two mechanisms that could explain the low compatibility between AHB and *V. destructor* in Mexico: (1) grooming behavior appeared significantly more intensive in AHB colonies, but was nevertheless ineffective; (2) EHB removed 8.03% of the infested brood, while AHB removed 32.46%, especially between 5 and 7 days post-capping. Though the cost of removing infested brood was not different between subspecies, the result, in terms of the amount of removed infested brood, was significantly higher for AHB. For both bees, there is thus a real cost, since removing a pupa results in a lower number of adult bees. We discuss the possibility that the removal of infested brood corresponds with a threshold above which the cost of removal becomes greater than the benefit.

***Apis mellifera* / *Varroa destructor* / parasitology / costs and benefits / behavioral resistance**

1. INTRODUCTION

The expression of a behavior, and the threshold above which it can occur, are difficult items to predict. The main reason is that decision-making generally depends on intricate genetic and environmental factors, both of which are poorly evaluated and modeled (Schmid-Hempel, 1986). In the particular case of host-parasite relationships, there are two lines of defense against parasites and pathogens. The first concerns behavioral defenses (e.g., grooming, avoidance of infected congeners) whereas the second concerns immune defenses (constitutive or adaptive). The benefit of behavioral resistance can be evaluated quite easily (for example, by measuring parasite death or decrease in prevalence). Conversely, the cost of behavioral resistance is difficult to quantify, since the cost is a loss of energy by performing tasks associated with resistance instead of normal tasks (Antonovics and Thrall, 1994). Such quantitative data are currently lacking, and the best existing data may be estimations as rough as evaluating water losses while performing behaviors associated with resistance (Hart, 1994).

Social insects are good models to estimate the costs and benefits of host resistance, provided that a macroscopic view of the population is adopted; i.e. the colonies are viewed as "individuals" within populations

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(see Schmid-Hempel, 1998, p. 204). The advantage of this approach, comparable to the theory of the superorganism (Moritz and Southwick, 1992), is that it is easy to determine how many individuals within a colony do spend time, die, or are killed while performing behaviors associated with resistance. Discrete data, appropriate only for quantifying cost, can hence be obtained using social insects. In particular, social insects may permit researchers to verify theoretical arguments stating that resistance does not pay below a certain parasite prevalence, because the expected costs exceed the expected benefits. The best strategy, under these models, would be to accept the damage caused by the parasites, defined as "tolerance" (Jokela et al., 2000). On the contrary, above a certain threshold, some defenses should be employed (Schmid-Hempel, 1998, p. 265).

Resistance should be considered here as a component of host-parasite compatibility. We define compatibility as the degree to which a host-parasite combination permits the development of the parasite, thus taking into account factors of both host and parasite that are able to affect their relationship (Ebert, 1994). A particular host-parasite combination can be compatible if it permits the development of a parasite, or incompatible if it does not permit its development (Toft and Aeschlimann, 1991). According to Minchella (1985), the level of compatibility depends on passive resistance, better called unsuitability, and on active resistance by the host against the parasite. "Unsuitability" is thus a host quality inhibiting parasite development, regardless of the host defenses (Lie et al., 1977). Resistance is an adaptive response of the host, the goal of which is to actively counteract the parasite's development. Contrary to unsuitability, resistance implies a cost for the host (Frank, 1992; Langand et al., 1998). In turn, "tolerance" is the acceptance of damage due to parasites (Jokela et al., 2000).

A very well-known model that may help to quantify costs and benefits of behavioral resistance is the combination formed by the honey bee *Apis mellifera* L. (European and African subspecies) and the parasitic mite *Varroa destructor* Anderson & Trueman in Mexico. Parasitism by genus *Varroa* was originally restricted to the Asian bee *A. cerana* Fabr., but the mite has extended its range onto the European-African bee *A. mellifera* in the 1920's. Since then, it has invaded almost every continent, being highly compatible and pathogenic to the bees in most countries. The mite was introduced to Uruguay in 1971 from Japan, and was found in Mexico in 1992. In the equatorial and tropical parts of the American continent, *V. destructor* has infested the Africanized honey bee (AHB), a hybrid of the European honey bee (EHB) subspecies *A. m. mellifera* and *A. m. ligustica,* imported during the 17th century, and the African subspecies *A. m. scutellata,* imported in 1956. In Brazil, the AHB/*V. destructor* system appeared less compatible, since only a fraction of the mites was able to reproduce in AHB colony's worker brood (Ritter and de Jong, 1984). Interestingly, the incompatibility seems to be related to the particular strain of mites introduced to South America rather than to the host bee (Anderson and Trueman, 2000).

We previously described a long-term survey of EHB/*V. destructor* and AHB/*V. destructor* combinations in Mexico (Vandame et al., 2000). We concluded that the EHB/*V. destructor* system was just as compatible and pathogenic as in other parts of the world (with more than 6000 mites per EHB colony), whereas the AHB/*V. destructor* system appeared to be less compatible (with a maximum of 2500 mites per AHB colony). Contrary to Medina and Martin (1999), it appeared that such differences were independent of mite fertility, i.e. the difference in compatibility could not be explained by a difference in parasite infectivity (Guzmán Novoa et al., 1999; Vandame et al., 2000). This situation is consistent with Anderson and Trueman (2000) who showed a correlation between the extension of the Korean haplotype of *V. destructor* and high levels of mite reproduction (mites in Mexico actually belong to this haplotype; de Guzmán, pers. com.). In the present paper, we follow this investigation by focussing on two mechanisms of behavioral resistance that may explain the difference in compatibility: grooming behavior and removal behavior.

1.1. Grooming behavior

Studies on the genus *Varroa*'s original host, *A. cerana*, revealed that adult bees perform a grooming behavior when mites are deposited on their thoraxes (Peng et al., 1987a; Bozic and Valentincic, 1995). Bees may engage in autogrooming behavior in which they brush their whole body with their legs, while twisting their abdomen. If the mite is not removed immediately, it may hide in the propodeum. The bee may begin dancing to induce allo-grooming behavior in which one or more bees look for the mite on the infested bee, pick up the mite with their mandibles, and eventually drop it on the hive floor. Correlatively, a high proportion of the dead mites collected on the hive floor are mutilated. However, it appears that most mites only change host bee, but are not killed or removed from the hive (Fries et al., 1996). Although the impact of this behavior on mite population dynamics remains unclear, it may partly explain AHB resistance.

1.2. Removal behavior

Removal, or hygienic behavior, involves the detection and removal of diseased brood. This behavior has been shown to be a behavioral mechanism of resistance to the bacterial disease, American foulbrood (Rothenbuhler, 1964) and some honey bees also remove brood infested with *V. destructor*. In naturally infested *A. cerana* colonies, bees remove a significant proportion of the mite infested brood (Peng et al., 1987b). When brood is artificially infested, bees remove only infested worker brood but not infested drone brood (Rath and Drescher, 1990). In one experimental infestation, *A. cerana* bees removed more than 50% of the larvae in cells infested with mites collected from *A. mellifera* colonies (Rosenkranz et al., 1993). But if introduced mites had been washed with ethanol and pentane, bees detected and emptied only 5% of infested cells (though a further 22.5% of cells were opened, and after removal of the mites, the cells were recapped). This suggests that mite odor permits the bees to detect and remove infested brood. Although *A. mellifera* workers remove less than 10% of cells artificially infested with mites collected in *A. cerana* colonies, they may remove a higher percentage of mites collected from *A. mellifera* colonies (reviewed in Spivak, 1996; Spivak et al., 1998; Boecking and Spivak, 1999). Such removal behavior could explain the low compatibility between AHB and the mite. Vandame et al. (2000) showed that the ability to resist *V. destructor* by honey bees was dependent on mite density, implying the existence of a threshold in the expression of removal behavior. They also observed a higher mortality of the population of brood mites in Africanized than in European colonies, suggesting the importance of removal behavior as an incompatibility factor.

In this experiment, we tested whether grooming or removal behavior significantly reduce the compatibility of *V. destructor* in AHB colonies. Based on the results, we attempted to quantify the costs and benefits of the behaviors to the bees.

2. MATERIALS AND METHODS

2.1. Site of observations and selection of honey bees

The experiments were conducted at the Colegio de Postgraduados, near the city of Córdoba (18^{o}53'N; 96^{o}56'W), state of Veracruz, Mexico, from August to November 1995. This area has a humid tropical climate, with annual rainfalls near 3000 millimeters and mean annual temperature of about 23 ^{o}C. Africanized honey bee (AHB) and European honey bee (EHB) colonies were selected from among a stock of colonies that had not been chemically treated against *V. destructor* for at least 2 years. This selection was based on discriminant analysis of bee morphology described by Daly and Balling (1978). The set chosen required measuring four characters of the right wing of 10 bees (i.e., forewing length and three angles between forewing veins). The colonies were selected for being extremely European or Africanized according to these measures, thus excluding intermediate degrees of hybridization between AHB and EHB.

2.2. Grooming behavior

To quantify the grooming capacities of EHB and AHB subspecies, we built two single-frame glass-walled observation hives (50 × 35 × 5 cm). One hive contained a frame from an EHB colony containing all-stages of brood, about 2500 European bees and a queen. The other hive contained a frame of brood from an AHB colony, 2500 Africanized bees and a queen. The hives were placed in an observation room with an exit to the outside for foraging. In addition, a brood frame, free of bees, was collected from a strongly infested hybrid colony. This frame was placed in an incubator overnight (32 °C, 70% RH), thus permitting adult bees carrying mites to emerge. Mites were collected, marked with queen-paint, and replaced on bees in the incubator for 24 h, so they would lose most of the paint odor. In a preliminary experiment, we verified that the odor of paint on the mite, after 24 hours, did not elicit any bee reaction. In this earlier experiment, 10 marked and 10 unmarked mites were deposited on bee thoraxes, and we measured the duration of auto-grooming. It lasted 167 ± 42 seconds for marked mites and 143 ± 61 seconds for unmarked mites, which was not significantly different (9 *df*; $P > 0.05$). Thus in the present experiment, we considered that mite painting had no effect on a bee's behavior.

A marked mite was collected with a fine wet brush and deposited on the thorax of a bee in an observation hive. Preliminary observations revealed that the strongest reactions of the bees occurred during the first 2 minutes. Thus the deposited mites were carefully observed for eight minutes, divided into six periods of time (0"–30", 30"–1', 1'–2', 2'–4', 4'–6', 6'–8'). Within each time-period, it was observed whether the bee receiving the mite performed auto-grooming behavior (brushing its whole body with its legs, or twisting its abdomen) or received allo-grooming behavior (by dancing, thus inducing one or more bees to come and search for the mite). It was also observed whether the mite changed bee or was dropped onto the hive floor. This experiment was repeated 10 consecutive times, each time with a different bee (for a total of 100 minutes). These sets of observations of 10 mites were repeated five times (i.e., with 50 mites) in each of the two hives. Then the frames were replaced in the original hives. The whole experiment was repeated three times, each time with a different EHB and AHB colony. The triplicate was thus based on the observation of 150 mites in each bee subspecies. Two hours after depositing the last mite, each hive was carefully checked, observing the bees one by one, to find mites and determine if they were marked, and to count the mites remaining on the bees and the mites lying on the floor.

In addition, 150 dead mites were collected three times, at two month intervals, on the hive floor of the field colonies of each bee subspecies. From each bee subspecies, 450 mites were checked by microscope, to detect any mutilation. Numbers of mutilated mites were compared using a χ^2 test.

2.3. Removal behavior

Removal behavior was observed in colonies containing naturally infested brood. Six colonies of similar strength were selected (three EHB and three AHB). Inside each colony, a 140 cm^2 brood zone, containing between 500 and 600 cells, well provided with L5 larvae was marked. Marked zones were checked every day. Following a previous experiment, the capping-duration was estimated to be 11.6 days for both subspecies. Daily observations made during 10 days permitted the detection of any early cell uncapping. When uncapped early, cells were checked to determine if the bee larva or pupa was apparently alive or obviously dead (translucent larva, black or headless pupa, etc.) and if the cell was apparently uninfested or obviously infested (presence of mites or faeces on cell walls). On the 11th day after capping, remaining capped cells were uncapped to determine if they were infested or uninfested. The ratio of removed dead brood to total brood, the ratio of apparently healthy removed brood to total brood, and the ratio of obviously infested removed brood to total infested brood were determined.

3. RESULTS

3.1. Grooming behavior (Fig. 1)

Over the nine days of the experiment, 150 mites were observed in each bee subspecies. When receiving a mite deposited on the thorax, 57% EHB and 80% AHB immediately auto-groomed, though less intensely than previously reported (Peng et al., 1987a). Though active allo-grooming behavior was observed, the bees were never seen to chew or catch the mite. After eight minutes of observation, nearly no bees performed further grooming behavior. The number of mites changing bee remained always low, and the number of mites falling off bees (the only effective result) was extremely low. Two hours after observation of the last mite, the number of mites remaining on bees was 139 in EHB colonies (9 mites found on hive floor, 2 mites not found) and 134 in AHB colonies (10 mites found on floor, 6 mites not found). Though there was a more intense reaction of AHB to the deposition of the mites, only a maximum of 11% of observed mites was definitively removed from bees, vs. a maximum of 8% in EHB.

Examination of dead mites collected on the hive floor of field colonies (Tab. I) showed that significantly more mites had injuries in AHB than in EHB colonies ($\chi^2 = 5.17$; $P < 0.05$). However, the observed percentages were far lower than the 30% measured on resistant

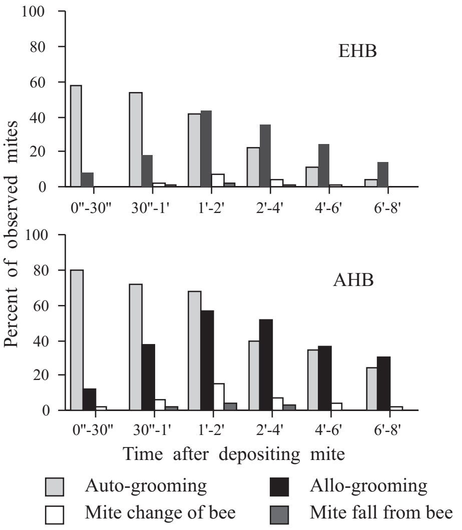

Figure 1. Grooming behavior. The individual behavior of 150 EHB and 150 AHB was observed for 8 minutes divided into six time periods after a mite was deposited on the thorax of the bee. The percent of bees performing auto- or allo-grooming behavior and the percent of mites changing host or falling from bees are shown.

Table I. Percent of intact mites or mutilated mites (on pretarsus of first pair of legs; first pair of legs; other body parts) of 450 mites collected in the debris of EHB and AHB field colonies and observed under a binocular microscope.

	EHB	AHB
Intact mites	90.6	85.1
Pretarsus 1	2.2	4.9
Leg 1	3.8	5.3
Others	3.4	4.7

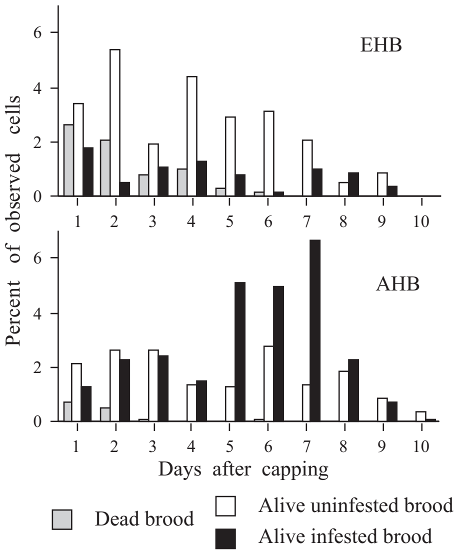

Figure 2. Removal behavior of mite infested brood. Daily observations were made for 10 days after cell capping on 1716 brood cells of EHB colonies (42.19% brood infestation) and 2367 brood cells of AHB colonies (24.29% brood infestation). On each day, the percent removal of dead brood of the total amount of brood, the percent removal of healthy brood of the total brood, and the percent removal of infested brood of the total infested brood were calculated.

A. cerana or even than the 12% on non resistant *A. mellifera* in China (Fries et al., 1996).

3.2. Removal behavior (Fig. 2)

During the 10 days after capping, 1716 cells were monitored in three EHB colonies, vs. 2367 cells in three AHB colonies. The level of brood infestation by *V. destructor* in the three EHB colonies was 42.2%, vs. 24.3% in AHB colonies, which was significantly different (2 *df*; t = 5.15; $P = 0.036$). As shown in Table II, there was variability between colonies of the same subspecies, as an effect of the small sample size. Nevertheless, the data were pooled for the rest of the analysis, to detect tendencies that may generate further discussion.

Within five days after capping, EHB removed 7.0% of brood observed to be dead (infested or not) vs. 1.6% for AHB, which was significantly different (2 *df*; t = 5.03; $P = 0.037$).

High levels of errors were made by the bees, since EHB also removed 24.7% of apparently live and uninfested brood, vs. 17.5% for AHB (2 *df*; t = 1.28; $P = 0.328$). Since the proportions of infested brood were 42.2% and 24.3% respectively, of total alive brood (i.e., excluding 7.0% dead brood for EHB and 1.6% for AHB),

Table II. Calculation of costs and benefits of removal behavior in three EHB and three AHB colonies, and average for each subspecies (bold type). Upper part of the table shows raw data of brood observed to die, to be infested, and to be removed (infested or not), during ten days of observations. Middle part shows calculations of the same data, including brood that was not removed (the data for each colony hence amount 100%). Lower part shows a synthesis of calculations. See the results section for statistical comparisons.

Data collected	EHB-1	EHB-2	EHB-3	**EHB**	AHB-1	AHB-2	AHB-3	**AHB**
Dead	4.8%	7.1%	9.2%	**7.0%**	0.9%	2.2%	1.6%	**1.6%**
Infested	44.2%	41.5%	40.8%	**42.2%**	19.4%	27.5%	26.0%	**24.3%**
Infested removed	5.7%	8.0%	10.4%	**8.0%**	28.4%	35.3%	33.7%	**32.5%**
Uninfested removed	11.4%	26.4%	36.3%	**24.7%**	12.8%	21.1%	18.6%	**17.5%**

Data calculated	EHB-1	EHB-2	EHB-3	**EHB**	AHB-1	AHB-2	AHB-3	**AHB**
Dead	4.8%	7.1%	9.2%	**7.0%**	0.9%	2.2%	1.6%	**1.6%**
Infested not removed	39.7%	35.5%	33.2%	**36.1%**	13.8%	17.4%	16.9%	**16.2%**
Infested removed	2.4%	3.1%	3.8%	**3.1%**	5.5%	9.5%	8.6%	**7.8%**
Uninfested removed	6.0%	14.3%	19.5%	**13.3%**	10.2%	15.0%	13.6%	**13.1%**
Uninfested not removed	47.1%	40.0%	34.2%	**40.5%**	69.7%	56.0%	59.3%	**61.5%**

Synthesis	EHB-1	EHB-2	EHB-3	**EHB**	AHB-1	AHB-2	AHB-3	**AHB**
Total costs	8.4%	17.4%	23.4%	**16.4%**	15.7%	24.5%	22.2%	**20.8%**
Total benefits	5.7%	8.0%	10.4%	**8.0%**	28.4%	35.3%	33.7%	**32.5%**

the brood opened erroneously represented 24.7% × (100 – 42.2%) × (100 – 7.0%) = 13.3% of total brood for EHB, and 17.5% × (100 – 24.3%) × (100 – 1.6%) = 13.1% of total brood for AHB, which was not significantly different (2 *df*; t = 0.13; *P* = 0.910).

Concerning the amount of brood removed that was infested with mites, EHB removed only 8.0% live infested brood, vs. 32.5% for AHB. The difference was highly significant (2 *df*; t = 17.29; *P* = 0.003). Since infested brood constituted 42.2% total brood in EHB, vs. 24.3% in AHB, the infested brood was nearly twice as difficult to find in AHB than in EHB colonies. Most of the 32.5% of cells were removed by AHB between the 5th and 7th days post-capping (Fig. 2). More than 30 infested cells were observed while pupae were being removed by bees. It was evident that bees never looked for the mite, and did not pick it up; hence the removed mother mites were probably able to start a new reproductive cycle.

These data enabled us to calculate the exact cost of the removal behavior. EHB removed 8.0% of live, infested brood, which represented 42.2% of total brood; excluding 7.0% dead brood from the calculation, the cost was thus 8.0% × 42.2% × (100 – 7.0) = 3.1% of total brood. AHB removed 32.5% of infested brood, which represented 24.3% of total brood; excluding 1.6% dead brood of the calculation, the cost was thus 32.5% × 24.3% × (100 – 1.6%) = 7.8% of total brood. Such costs were significantly different (2 *df*; t = 4.96; *P* = 0.038). If the cost of errors is included by adding the cost of removing apparently live, uninfested brood (13.3% and 13.1% respectively for EHB and AHB colonies) to the cost of removing infested brood (3.1% and 7.8%), the total cost of removal (respectively 16.4% and 20.8%) appears only 26.7% higher for AHB than for EHB colonies, which was not a significant difference (2 *df*; t = 1.56; *P* = 0.259). However, the proportion of correctly removed to total removed brood remains much lower for EHB colonies

(3.1% / 16.4% = 19.2%) than for AHB colonies (7.8% / 20.8% = 37.3%).

Above all, for AHB, the cost of removal was thus higher than for EHB (20.8% vs. 16.4% of total brood), though the difference was not significant. The benefits, in terms of the percentage of infested brood that was removed was also higher (32.5% vs. 8.0% of infested brood), showing a highly significant difference.

4. DISCUSSION

The development of *V. destructor* populations is very low in Africanized honey bee colonies in Mexico as it is in Brazil, (Guzmán Novoa et al., 1999; Vandame et al., 2000). However the reason for the low levels of mites in AHB colonies in Mexico is not due to low mite fertility, which reinforces the hypothesis that low mite reproduction may be associated with the genetic strain of the mite rather than the strain of the bee (Anderson and Trueman, 2000; Vandame et al., 2000). The aim of this paper was to examine two behavioral factors which may limit the mite population development in AHB colonies in Mexico. Due to the techniques chosen and the long observation times they required, the sample size was rather small. Thus our discussion is rather speculative, and intends more to generate new hypotheses and propose new research focuses than to propose definitive interpretations of the data.

4.1. Behavioral resistance

Grooming capacities were slightly higher in AHB than in EHB, since the response to *V. destructor* deposited on the thoraces of the bees provoked stronger and longer response in AHB colonies. Hence AHB spent time and energy in attempting to remove the mites. Thus there was an apparent cost to this behavior making it a factor of resistance. Nevertheless, the auto- and allo-grooming responses were never comparable in intensity with observations in *A. cerana* colonies, since the bees never twisted their abdomen or performed the grooming dance reported in that bee species (Peng et al., 1987b). Only a maximum of 11% of the observed mites were definitely removed by AHB, vs. a maximum of 8% in EHB, which is an insignificant difference. Thus it seems very unlikely that grooming behavior may explain the low compatibility between AHB and *V. destructor.*

However, stronger differences were detected when we tested the capacities of bees to remove infested brood: EHB removed only 8.0% of infested brood (i.e., 3.1% of total brood), vs. 32.5% (i.e., 7.8%) for AHB. In cases where a pupa was removed, the mother mite remained alive and may have started a new reproductive cycle, but all her offspring in the opened cell must have soon died because they are unable to feed on adult bees. Under laboratory conditions, a mother mite is able to perform up to 7 reproductive cycles during its life span, laying thus a total of 35 eggs (de Ruijter, 1987; Donzé and Guérin, 1994). It has been shown, however, that under natural conditions, only 14% of mother mites actually perform three reproductive cycles (Fries and Rosenkranz, 1996). Killing the offspring of one brood cycle hence reduces considerably the fitness of a mother mite.

We report a high proportion of erroneously removed brood, which was similar in both races of bees (13.3% of total brood in EHB colonies vs. 13.1% in AHB). This observation may be a function of excessively high levels of the cue that indicates to the bees that the brood is infested, leading to some imprecision by the bees. The cue could be the odor of mites, faeces or offspring. The proportion of brood correctly removed to total brood removed was nevertheless much higher in AHB than in EHB colonies (37.3% vs. 19.2% of total removed brood). This demonstrates that AHB are more effective at removing infested brood than EHB, and that EHB make more errors than AHB. Both these aspects tend to confirm that removal behavior may be an actual factor for the low compatibility between AHB and the mites (Spivak and Reuter, 1998, 2001). Our data should be interpreted with care, since they concern a small number of colonies, and in particular, were performed on colonies with different infestation levels. However, the technique chosen demonstrated the existence of removal behavior specifically directed against *V. destructor* under natural conditions. Further studies could be done with other techniques to improve removal capacities (e.g., brood freezing or artificial infestation) but this requires first the determination of a good correlation of such techniques with removal of naturally infested brood.

The effective removal behavior reported here fits well with previous observations (Vandame et al., 2000) in two aspects. (i) We showed previously that the intrinsic rate of natural increase (*r*) of *V. destructor* was not different in EHB and AHB colonies, suggesting that the factor(s) accounting for resistance was not expressed at low prevalence, but rather had a density-dependant nature. Hence we may hypothesize here that the bees accept a given prevalence (i.e., percent of infested larvae) above which they start removing infested brood. Such hypothesis may fulfill the density-dependence characteristics previously evidenced. In addition, this kind of threshold has been predicted theoretically (see Schmid-Hempel, 1998, p. 267). (ii) We also have shown that the death rate of phoretic mites was not different between AHB and EHB colonies, but that the death rate of mite populations in brood could be twice as high in AHB colonies compared to EHB colonies. Since the death of removed mites accounts partly for brood mite mortality, the higher removing abilities of AHB fits well with a higher mortality in brood AHB mites population. Complementarily to these observations, further experiments based on artificial infestation of brood would permit clarification of why infested brood is removed by AHB so precisely between 5 and 7 days after capping. This period could indicate that the detection of infested brood is more likely for bees at this time, for some reason, e.g. alert pheromone emission by the pupa, temperature lowering of the pupa interpreted as a disease symptom by adult bees, fecal accumulation reaching a size that makes it detectable by adult bees (Rehm et al., 1989; Donzé and Guérin, 1994; Boecking and Spivak, 1999).

4.2. Quantifying the cost of resistance

The whole reproductive cycle of *V. destructor* occurs inside the bee brood cell, during the pupal period of the bee (see Martin, 1994; Donzé and Guérin, 1994). It is worth making explicit here the cost of parasitism for a bee pupa, because it will further permit a prediction of whether behavioral resistance should be expressed or not by the bees. *V. destructor* actually rarely kills its host pupa, since it is not highly pathogenic per se (unless it vectors a virus). As pointed out by Ball (1985), the death of the pupae (and consequently of the mites) is frequently observed at an infestation of four or more foundress mites per pupa. According to previous observations (Vandame et al., 2000), in a tropical climate where the bees have continuous brood rearing, such levels of infestation occur in only 0.55% of infested cells (16 out 2898). Thus in the absence of removal behavior expressed by adult bees, an infested pupa survives up to the imaginal stage, and supports mite reproduction. In turn, an adult bee that was infested by the mite during its ontogenesis is affected in terms of weight (from 8% reduction if infested by 1 mite, up to 25% if infested by 4 or 5 mites; Marcangeli et al., 1992) or life span (reduction of at least 20%; de Jong et al., 1983).

As to the cost of the removal behavior, both races of bees spent time and energy removing brood which destroyed part of their brood. Because the pupae inside would not be killed by the mites if the brood was not removed, removing – i.e. killing – a pupa that would otherwise survive (though for a reduced life-span) is thus actually a cost for the colony. For both EHB and AHB, removal of infested brood has thus a real cost, so this behavior is fully a resistance trait. For AHB, the cost is actually slightly higher than for EHB (20.8% vs. 16.4%), though the difference was not significant, but the result, in terms of removed infested brood was very significantly higher (32.5% vs. 8.0%). These results raise two main questions.

1. AHB remove 32.5% of infested brood, but why *only* this level? A first factor that may determine a limitation in removal is that infested cells are probably unequal in the intensity of the cue that reveals the infestation to the bees, and bees detect only the cells emitting the most intense signal. Another factor is that, depending on their genotype (selected or not for removal behavior), the bees present different olfactory and behavioral response thresholds to odors of diseased brood (Masterman et al., 2001). Inside a colony, there is actually a division of removal labor, and individual bees can perform different components of this behavior

(uncapping and removing) with higher frequency depending on the genotypic composition of the colony (Arathi and Spivak, 2001). Both these sources of variation (cue intensity and expression of behavior) may account for limited abilities of the bees to detect mites reproducing in their brood.

A more speculative hypothesis relates to the fact that a host has to make a trade-off between its own strength and the impact of the parasite (Hart, 1994; Combes, 1995). That is, the host may lose more in an all-out struggle with the parasite than it loses by tolerating the current level of parasitism (Zahavi and Zahavi, 1997). In a recent paper, Jokela et al. (2000) modeled the balance between resistance (energy allocation to defense, implying a loss in fitness due to defense itself) and tolerance (no allocation to defense, implying a loss in fitness due to parasite attack). They showed in particular that an increase in expected damages can select for investment in defense, but only up to a point after which tolerance may become the superior strategy. At this point, the fitness difference between defending and non-defending individuals has decreased to zero.

The *Apis / V. destructor* case reported here could constitute an illustration of this model. It could well be that at low mite prevalence, the best strategy would be to tolerate parasitism, and above a threshold of prevalence, the best strategy would be to remove part of infested brood. But removing a high proportion of infested brood may have an excessive cost for the bees, in terms of killed brood and potential adult bees, and would have an unendurable impact on the colonies. An adaptation of existing *V. destructor* population dynamics models (Fries et al., 1994; Calis et al., 1999) could show if such effectiveness can be enough to provoke a decrease of the mite population; if so, it would strengthen the hypothesis that bees actually make a trade-off between brood killing and *V. destructor* population size. An experimental setting of prevalence by artificial infestation of brood would also make it possible to determine the existence of a threshold for removal behavior expression.

In the case of EHB, the level of infested brood removed was only 8.03%. This may be first because they have less ability than AHB to detect and remove infested brood, as will be discussed further. This may also be because the infestation level in EHB colonies was high at the time of experiments which may saturate the cue that signals an infested brood cell to worker bees which would prevent them from accurately detecting the infested cells, and possibly lead them to remove uninfested cells. This could be the reason why we saw EHB remove as much as 24.7% of uninfested brood. Alternatively, at high infestation levels, there could be an habituation of worker bees to some level of the infestation cue, that diminishes their response to such cue.

In a synthetic view, it seems that there are two limits to removal behavior: (i) the removal level a colony may bear; (ii) the percentage of infested brood that the bees can accurately detect. In our case, it was striking that the overall cost of removal was rather similar in EHB and AHB colonies (16.42% vs. 20.81% respectively). This level may represent the first limit, i.e. the percentage of brood that a colony can kill for disease control without lowering too much its own fitness and survival. The distinct difference between the races is the amount of brood they correctly remove (19.2% vs. 37.3% respectively). This amount may represent the second limit, i.e. the percentage of infested brood that worker bees can accurately detect. Such a limit may be due either to the accuracy in detecting diseased brood that is characteristic of each bees' subspecies, or to the rate of errors resulting from the infestation level. In our case, the combination of the first and second limits would provide the actual benefits to bees (3.1% vs. 7.8% of total brood respectively, i.e., 8.0% and vs. 32.5% of infested brood). A simple way to test this hypothesis would be to generate various levels of infestation within colonies belonging to a single bee strain, and measure if above all, all of them remove a similar brood percentage (first limit), and if inside this percentage, the level of errors increases with the level of infestation (second limit).

2. In Mexico, the AHB/*V. destructor* system was established for the first time in 1992, thus it appears unlikely that AHB have been selected in this country for removal capacities before the start of our observations in 1994. A simple explanation for the occurrence of this behavior

may be that all the bees have had to defend against pathogens in brood, whatever the species they belong to or their geographical origin; hence, removal behavior could belong to their genetic pool. This would mean that this behavior is a general one, that may be applied in any case of brood disease. Another explanation emerges when the host evolution previous to the encounter with the parasite is considered. It is well established that all European and Africanized honey bee subspecies diverged from an east-Mediterranean common ancestor (Garnery et al., 1992). Yet the main challenge for survival of European subspecies has been the climate. For the African subspecies it has been the confrontation with predators and parasites (Seeley, 1983); to date, 160 mite species are known to be associated with tropical honey bees (Roubik, 1992). The defense against predators has been an increase in aggressiveness, and the main defense against brood parasitism could well be the removal behavior (Ruttner, 1988). Thus we can hypothesize that the removal behavior reported in the present paper for AHB mainly constitutes a trait acquired by African bees during their evolution, prior to their encounter with *V. destructor.* Hence, such a trait would currently provide benefits for which it was not previously selected, which corresponds to the definition of an *exaptation* (sensu Rose and Lauder, 1996, p. 43).

Another speculation could be that European and US commercial honey bee populations (those that have been protected by man from dying from *V. destructor*) arose from human selection over centuries (Ruttner, 1988). Factors selected include low defensiveness, low level of nestmate discrimination and, may be as a side effect, lower resistance against parasitism. Thus, we hypothesize here that the bees usually classified as European (including those tested in our experiments, introduced from the Old World during the XXth century after selection by man) are mainly derived from anthropogenical selection. They are actually gentle, but also form compatible associations with parasites like *V. destructor.* On the contrary, feral bees, when they still exist (AHB that we tested can be considered as such), though less gentle, would still have intact resistance abilities. This hypothesis will be elaborated on in a further paper (Vandame et al., unpublished data).

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Résumé – Parasitisme chez l'abeille sociale *Apis mellifera* : quantification des coûts et bénéfices d'une résistance comportementale à l'acarien *Varroa destructor.* L'expression d'un comportement et le niveau à partir duquel il peut se manifester sont des sujets difficiles à prédire car les facteurs génétiques et environnementaux en jeu sont complexes. Le cas de la relation hôte-parasite peut fournir un bon modèle pour évaluer les coûts et les bénéfices de certains comportements, à condition de faire la distinction entre les divers composants de la compatibilité. Les abeilles européennes et africanisées sont deux sous-espèces d'*Apis mellifera* L. qui coexistent au Mexique ; les premières forment une association fortement compatible avec l'acarien *Varroa destructor* Anderson & Trueman, les secondes une association moins compatible. La compatibilité relativement faible entre les acariens et les abeilles africanisées au Mexique ne peut pas être attribuée à la faible fertilité de l'acarien, contrairement à ce qui se passe au Brésil. Nous examinons ici deux mécanismes qui pourraient expliquer la faible compatibilité entre les abeilles africanisées et *V. destructor* au Mexique, tous deux étant des facteurs de « résistance de l'hôte » : (1) le comportement de toilettage semble significativement plus intense chez l'abeille africanisée (Fig. 1) mais reste néanmoins inefficace et le taux d'acariens mutilés est le même chez les deux sous-espèces (Tab. I), (2) les abeilles européennes éliminent 8,03 % du couvain infesté, tandis que les abeilles africanisées en éliminent 32,46 %, particulièrement entre le 5^{e} et le 7^{e} jour après l'operculation (Fig. 2). Bien que le coût d'élimination du couvain infecté ne soit pas différent d'une sous-espèce à l'autre, le résultat, en terme de quantité de couvain infesté éliminé, est significativement plus élevé chez l'abeille africanisée (Tab. II).

Pour les deux sous-espèces il y a donc un coût réel, puisque l'élimination d'une nymphe entraîne un nombre réduit d'abeilles adultes. Ce comportement peut donc réellement être considéré comme un mécanisme de résistance. Nous discutons la possibilité que l'élimination du couvain infesté corresponde à un seuil au-dessus duquel le coût d'élimination devient supérieur au bénéfice. Un tel seuil a été prédit par la théorie mais nécessite encore d'être vérifié par l'expérience.

Nous supposons que la différence dans l'élimination du couvain infesté reflète le fait que les abeilles africanisées proviennent des tropiques, où elles ont été sélectionnées sur la défense contre les prédateurs et les parasites, tandis que les abeilles européennes proviennent de la zone tempérée, où elles ont été sélectionnées sur la survie à l'hiver et non sur la défense. Autrement, cela pourrait être dû au fait que les abeilles européennes ont été soumises à la sélection par l'homme pour la douceur, ce qui a pu entraîner comme effet secondaire une faible résistance aux acariens parasites.

***Apis mellifera / Varroa destructor* / parasitologie / résistance comportementale / coûts et bénéfices**

Zusammenfassung – Parasitismus bei der sozialen Biene *Apis mellifera*: Quantifizierung der Kosten und des Nutzens von Resistenzverhalten gegen *Varroa destructor* Milben. Die Äußerung eines Verhaltens und die Schwelle oberhalb derer es auftreten kann, sind schwer vorhersagbar, da komplizierte genetische Faktoren und Umweltfaktoren daran beteiligt sind. Ein Wirt-Parasit-Verhältnis könnte ein gutes Modell abgeben, um die Kosten und den Nutzen einiger Verhaltensweisen abzuwägen, vorausgesetzt, die verschiedenen Bestandteile der Kompatibilität werden unterschieden.

Europäische (EHB) und Afrikanisierte (AHB) Honigbienen sind zwei Unterarten von *Apis mellifera* L., die beide in Mexico vorkommen. Erstere hat eine stark kompatible Beziehung zu *Varroa destructor* Anderson & Trueman, letztere eine weniger kompatible. Im Gegensatz zur Situation in Brasilien kann die relativ geringe Kompatibilität zwischen Milben und AHB in Mexico nicht auf eine niedrige Milben-Fertilität zurückgeführt werden. Wir untersuchen hier zwei Mechanismen, die die geringe Kompatibilität zwischen AHB und *V. destructor* in Mexico erklären könnten. Beides sind Faktoren der Wirts-Resistenz: (1) Putzverhalten trat bedeutend intensiver in AHB Völkern auf (Abb. 1), blieb aber nichtsdestotrotz ohne Auswirkung und die Anzahl der verstümmelten Milben war in beiden Unterarten ähnlich (Tab. I); (2) EHB räumten 8,03 % der infizierten Brut aus, wohingegen AHB 32,46 % ausräumten, insbesondere zwischen dem 5. und 7. Tag nach der Verdeckelung (Abb. 2). Obwohl die Kosten für das Ausräumen infizierter Brut bei beiden Unterarten gleich hoch sind, war die Menge der ausgeräumten infizierten Brut bei AHB signifikant größer (Tab. II). Für beide Bienen gibt es insoweit tatsächliche Kosten, als das Ausräumen einer Puppe die Anzahl schlüpfender erwachsener Bienen verringert. Daher kann dieses Verhalten als ein Resistenz– Mechanismus gedeutet werden. Wir diskutieren die Möglichkeit, dass es für das Ausräumen infizierter Brut eine Schwelle gibt, ab der die Kosten für das Ausräumen größer werden als der Nutzen. Eine solche Schwelle wurde theoretisch vorhergesagt, muss aber noch experimentell bestätigt werden.

Wir vermuten, dass der Unterschied im Ausräumverhalten daher rührt, dass AHB aus den Tropen stammen, wo der Selektionsdruck auf Verteidigung gegen Beutegreifer und Parasiten lag, wohingegen EHB aus gemäßigten Breiten stammen, wo sie auf das Überleben der Winter und nicht auf Verteidigung selektiert wurden. Alternativ könnte es daher rühren, dass EHB durch den Menschen auf Sanftmut gezüchtet wurden, wodurch als Nebenwirkung eine geringere Resistenz gegen parasitäre Milben bedingt worden sein könnte.

***Apis mellifera / Varroa destructor* / Parasitologie / Kosten und Nutzen / verhaltensbedingte Resistenz**

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