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ORIGINAL RESEARCH ARTICLE

Uncapping of pupal cells by European bees in the United States as responses to *Varroa destructor* and *Galleria mellonella*

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Summary

We investigated the uncapping of pupal cells by honey bees in the United States as responses to infestation with *V. destructor* and *G. mellonella*. In a group of 15 colonies, we counted the number of uncapped cells with pupae, and estimated the total sealed brood area. At the same time, the infestation by *V. destructor* and evidence of wax moth activity were measured in uncapped pupal cells, in cells immediately adjacent (neighbours), and in cells along linear transects. The relative amount of uncapped pupal cells (uncapped pupal cells/total sealed cells) increased with infestation (linear regression R^2 = 0.64, slope = 0.16). Varroa mite infestation of uncapped cells (30%) significantly exceeded that of neighbours (14%) and also that of transect cells (12%), but infestations of neighbour and transect cells were similar. The relationship between infestation of uncapped cells and that of other cells (neighbour or transect cells) had a slope significantly higher than one, suggesting that discrimination of infested cells increases with overall colony infestation. Frequencies of wax moth activity (presence of larvae, frass, tunnelling and webbing) were highest in uncapped cells (33%), lower in neighbour cells (21%) and extremely low in transect cells (4%). We followed the opening, removing and resealing of pupal cells every 24 h in a colony with one of the highest infestations with varroa mites and with one of the lowest levels of wax moths, and in a second colony with the opposite infestation levels. Many opened or partly removed cells were found in a different condition, suggesting a dynamic process under conditions of high infestation.

Keywords: uncapping, hygienic behaviour, *Apis mellifera, Varroa destructor,* Galleria mellonella

Introduction

Adult honey bees have been shown to detect a variety of problems in brood cells and may respond with uncapping of cells and removal of affected individuals. The first reports of diseases or pests inducing this hygienic removal include: American foulbrood (Park, 1937), chalkbrood (Gilliam *et al*., 1983), varroa mites (Peng *et al*., 1987) and small hive beetle larvae (Ellis et al., 2003). Larval activity by *Galleria mellonella* L. causes bees to open cell cappings over pupae ("bald brood"), but this is not necessarily followed by removal (Williams 1978). Physical damage by freezing (Taber, 1982) or piercing of larvae (Newton & Ostasiewski, 1986) can also initiate removal, and removal of freeze-killed brood has become a standard technique to quantify "hygienic behavior."

The recent interest in hygienic behaviour derives largely from its potential for effective control of *Varroa destructor.* Reports of hygienic removal of varroa mites cover wide geographic and taxonomic ranges. Selective removal of brood infested with mites has been shown in Apis cerana in China (Peng *et al*., 1987), in European *A. mellifera* in Germany (Boecking & Drescher, 1991) and in the USA (Spivak 1996), and in Africanized bees in Brazil (Corrêa-Marques & De Jong, 1998) and Mexico (Vandame *et al*., 2000). The detection and targeted removal of brood with reproductive mites (so-called "varroa-sensitive hygiene," VSH) has been shown to be the primary defensive mechanism of bees selected for "suppressed mite reproduction" (SMR) from a base population in the USA (Harbo & Harris, 2005).

One of us (Villa) noted numerous cells with opened cappings and exposed pupae in untreated "survivor" colonies in Louisiana. The colonies had not been selected for hygienic behaviour of freeze-killed brood or for specific resistance to varroa mites, but had survived longer than expected with densities of varroa at low to moderate levels. We looked for possible explanations for the uncapping of cells in these colonies and compared them with

Russian colonies and colonies of VSH outcrosses (formerly known as SMR). We investigated the causes for uncapped cells as a response to: (1) the fairly recent parasitic mite *V. destructor* and (2) infestation by larval *G. mellonella* which have coexisted with European bees for much longer. We also followed the time course of opening of cells, removing of pupae, and resealing of cells in a colony highly infested with varroa and in a colony with a high infestation of wax moth larvae.

Materials and Methods

Fifteen colonies from three origins were surveyed for the presence of uncapped cells with pupae still in them. Five of the colonies were local colonies initially exposed to varroa mites and not having received treatment within two to five years. Eight colonies were of Russian origin, and two colonies were headed by out crossed VSH queens. For each colony, all uncapped cells with pupae were counted and the total sealed brood area measured. Two to five brood frames containing uncapped cells with pupae were removed for inspection of three types of cells: (1) uncapped cells with pupae, (2) pupal cells immediately adjacent to the uncapped cells (potentially up to six neighbour cells per uncapped cell), (3) pupal cells along randomly chosen horizontal transects across both sides of two of the frames. Cells were examined under 5X magnification for signs of varroa presence (mites or feces) or wax moth activity (larva, frass, or tunnels).

The relationship between proportion of uncapped cells (number of uncapped cells with pupae/number of sealed brood cells) and the infestation of transect cells (proportion of cells infested with mites, showing activity of wax moth larvae, or both) was analyzed with linear regression. Percentages of infestation with either *V. destructor* or *G. mellonella* activity in uncapped cells, neighbour and line transect cells in each colony were compared with ANOVA using a random block design with cell type as treatment and colony as random block. Linear regressions were conducted between the infestations of the three cell types with each organism. Two Russian colonies were omitted from the last two analyses because samples had fewer than 20 uncapped cells. Sheets of transparent acetate film were used to mark unusual conditions of individual cells in two brood combs from each of two colonies at 0, 24 and 48 h. This allowed observations of daily changes in the opening of cells, removal of pupae, and resealing. One of the two colonies was the most highly infested with mites (54% in transect cells) and had no detectable activity of wax moths, and the other had a high infestation of wax moth larvae (11%) but low V. destructor infestation (1%).

Results

Of 582 uncapped cells with pupae that were examined in the 15 colonies, 24% contained evidence of *V. destructor* infestation (adults, immatures or feces), 29% showed evidence of *G. mellonella* activity (larvae, tunnels, frass or webbing), 4% had signs of both mite and wax moth larval activity, and 43% showed no apparent anomalies. The proportion of uncapped cells with pupae (number of uncapped cells/total number of sealed cells) significantly increased as overall infestation (varrroa mites, wax moths or both) increased (linear regression $R^2 = 0.64$, b = 0.16).

In 13 colonies in which the contents of more than 20 uncapped pupal cells were examined, the percentage of uncapped pupal cells with evidence of varroa mites (30% ± 5) was significantly higher ($P = 0.0002$) than that of adjacent neighbour cells with sealed pupae (14% ± 5). The infestation of neighbour cells was not different ($P = 0.64$) from that of pupae along linear transects in the same combs (12% ± 5). Aside from one local colony that exhibited a much higher level of infestation in uncapped cells with respect to their neighbours, different origins (Russian, local or VSH-outcrossed) did not show different patterns of infestation.

With respect to infestation or evidence of *G. mellonella* activity, uncapped cells with pupae had much higher levels than those of linear transect cells with pupae (33% ± 4 vs. 4% ± 4; $P < 0.0001$). Contrary to what was found for varroa mites, wax moth activity in neighbour cells (21% ± 4) was much closer to that of open cells (albeit significantly different, $P = 0.0038$) and much higher than transect cells ($P = 0.0001$).

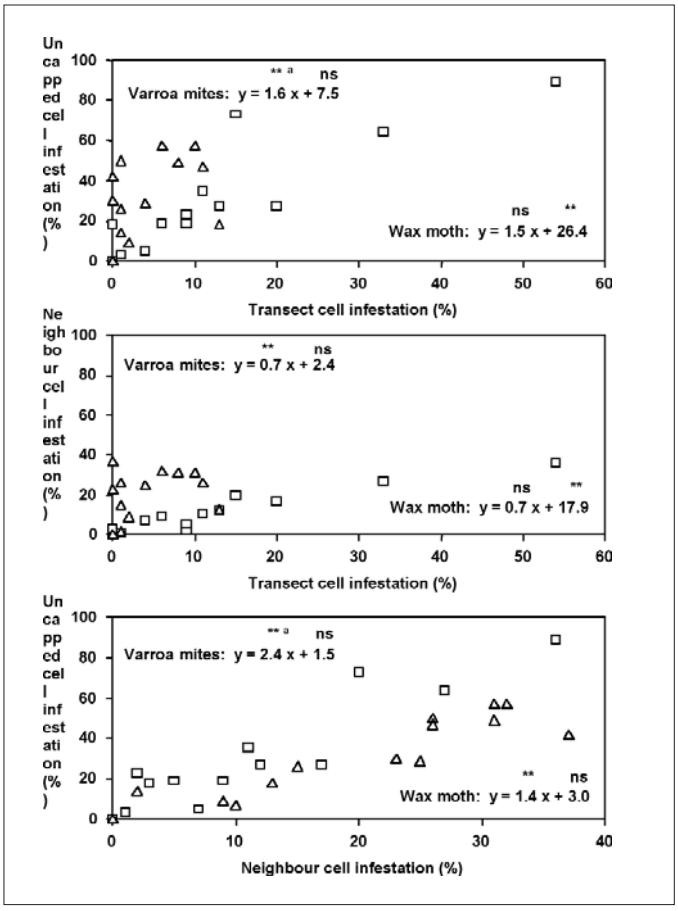

Fig. 2. Relationships between percentages of pupal cells with varroa mites (□) or with wax moth larval activity (△) in uncapped pupal cells, in cells along a linear transect, and in cells immediately adjacent to uncapped cells (neighbour) measured in the same combs in 13 colonies. The corresponding linear regression equations are shown for infestation with varroa and for activity with wax moths in each graph. Level of a significant difference from 0 for slopes and y intercepts is indicated (ns: $P>0.05$, *: $P<0.05$, **: $P<0.01$). Additionally, slopes significantly higher than 1 are indicated by "a."

Linear relationships between infestation of the three types of cells showed different patterns for the two parasites. For infestation with varroa mites, the three cell types were highly correlated (Fig 1). Furthermore, the infestation of neighbour cells tracked transect cells very closely, while the infestation of uncapped cells increased at a rate different from 1 with respect to both transect or to neighbour cells (Fig 1). For wax moth larvae, activity in transect cells was low compared to the values seen for varroa mites, but was higher in the other two types of cells (Fig 1). These differences lead to y intercepts different from 0 when comparing variables by linear regression. However, while the slopes relating infestations with varroa in the three types of cells showed slopes significantly different from 0, in the case of wax moth activity only the relationship between uncapped cells and neighbour cells showed a slope significantly different from 0. Observations every 24 h of cells that had been opened or had pupal contents partly removed indicated many transitions (Table 1). The colony most highly infested with *V. destructor* (54% in transect cells) had high numbers of uncapped cells, half of which remained uncapped after 24 h. A third of the uncapped cells were recapped, while most of those showing partly removed pupae were removed within 24 h. In contrast, a colony with high wax moth activity and low *V. destructor* infestation (11% and 1%, respectively in transect cells), showed a lower frequency of uncapping cells, no pupae in the process of being removed, and over half of the opened cells being recapped in 24 h (Table 1).

Discussion

The much higher infestation by *V. destructor* and more frequent evidence of activity of wax moth observed in uncapped cells, when compared to linear transects of cells, indicates that colonies in the USA from various origins were detecting the presence of these organisms. Even though bees exhibited a high degree of specificity towards both organisms, responses seemed to vary in different ways as infestation by the two organisms increased. There appeared to be a high detection ability for wax moth activity at any level of infestation, whereas for varroa mites the detection ability appeared to increase with the level of overall colony infestation (as indicated by slopes significantly higher than 1 between the dependent variable of uncapped cell infestation and either transect cell infestation or neighbour cell infestation).

When data collected on 10 Africanized colonies in Brazil by Corrêa-Marques and De Jong (1998) were analyzed, the average slope relating uncapped pupal cells and random cells is 2.3 (but because of higher variability, the 95% confidence limit still includes 1), again suggesting increased discrimination of varroa-infested cells with increasing infestation. Calculations from data presented by Vandame *et al* (2002) show that three colonies morphometrically identified as Africanized were uncapping infested brood at similar or higher rates than the colonies surveyed by us, while three European colonies showed no significant differences between the mite infestation of random cells and that of uncapped and removed cells. It appears that USA European colonies derived via artificial selection, natural selection, or both (VSH, survivor or Russian, respectively), can be as specific towards *V. destructor* as Africanized bees in Brazil and in Mexico.

Changes in the status of uncapped cells observed in two colonies with contrasting infestations of varroa mites and wax moth suggest that responses to the two parasites may also differ in temporal patterns. In one colony highly infested with varroa, the uncapping rate of pupae was high, about half of the uncapped cells remained opened, and partly removed pupal contents were completely removed within 24 h. In contrast, in a colony with low levels of varroa but high wax moth larval activity, uncapped cells tended to be resealed. The temporal patterns of uncapping, resealing, and removing need to be followed in more colonies to ascertain whether these trends are consistent.

Two approaches to improve the varroa-specific hygienic behavior of colonies have produced economically useful traits: selection for removal of freeze-killed brood (Spivak & Reuter, 2001) and selecting for selective removal of reproductive mites (Harbo & Harris, 2005). However, hygienic behavior towards freeze-killed brood may not correlate closely with hygienic

Table 1. Transitions every 24 h in the status of individual cells in two colonies during observations for 48 h. Colony 523 had the highest infestation of *V. destructor* (54% of pupal cells along a linear transect) and colony 103 one of the lowest (1%) but had one of the highest levels of activity of *G. mellonella* (11%). Cells categorized as chewed were found with partially removed pupae. No partly removed cells were observed in colony 103.

Col. no.	**Cells observed at beginning of 24 h periods as:**	**Transition every 24 hours (% of total cells at beginning)**			
		Same State	Recapped	Removed	Part. Removed
523	Uncapped (*n*=330)	51%	34%	11%	4%
	Part. Removed (*n*=25)	8%	0%	92%	0%
103	Uncapped (n=30)	10%	57%	33%	0%
	Part. Removed (n=0)	–	–	–	–

behavior towards varroa mites (Mondragon *et al,* 2005, Spivak, pers comm.). Additionally, while selective removal of reproductive mites may be the most effective control of mite growth, targeted removal of infested pupae may also decrease mite population growth (Vandame *et al,* 2002). Our findings suggest that other simpler approaches toward selection for resistance may be possible. Comparing the differences in infestation between uncapped pupal cells and transects of cells in colonies of different genotypes (or of uncapped cells and neighbour cells) could help discern possible genotypic influences. Differences in either the slope or the y intercept of regression lines could lead to the selection of groups showing higher selectivity towards *V. destructor.* Another approach might be to select individual colonies with infestations of uncapped cells much higher than suggested by a linear regression derived from a pool of colonies.

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