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Original article

Weight loss in drone pupae (*Apis mellifera*) multiply infested by *Varroa destructor* mites

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Abstract – Colony losses caused by varroatosis in the western hive bee, *Apis mellifera*, are mainly due to worker bee injury by *Varroa destructor* mites during preimaginal development. These effects have been examined repeatedly; however, little is known about effects on drones. Here we present the first exact data on individual drone weight loss, especially from the red eyed pupal stage onwards. The resulting reduction in weight of adult drones was related to the number of female mites (up to 20) that had invaded a brood cell. Weight loss was significant even if only one female mite was present. From several of the heavily infested brood cells adult mini-drones emerged.

Varroosis / multiple drone brood infestation / preimaginal weight loss / male fitness / *Apis mellifera* / *Varroa destructor*

1. INTRODUCTION

The breakdown of *Apis mellifera* L. colonies due to *Varroa destructor* Anderson & Trueman (2000) results mainly from worker brood infestation. Mite females can reproduce in worker cells of the western honey bee, and their feeding leads to pupal weight loss (De Jong et al., 1982). The parasitized adult bee emerges injured and with a reduced life expectancy (De Jong and De Jong, 1983). The effects of parasite load have been studied mainly in worker bees (Schneider and Drescher, 1987; Schatton-Gadelmayer and Engels, 1988).

In comparison, little has been recorded on drone injury. While *V. destructor* females are rarely found in queen cells (De Jong, 1990), drone brood of *A. mellifera* is much more heavily infested than worker brood (Gonçalves et al., 1982; Sulimanovic et al., 1982; Issa and Gonçalves, 1984; Schulz, 1984; Fuchs, 1990; Boot et al., 1995). The resulting damage to adult drones (Weinberg and Madel, 1985) and the decrease in the number of males from infested colonies available for mating (Rinderer et al., 1999) have been described.

The level of pupal infestation by *V. destructor* mites that causes a reduction in weight of individual drones has not been determined. We studied weight loss during preimaginal development in colonies with multiple brood infestation. Our results show that any parasitism affects the weight of adult drones and may reduce their fitness. However, drones often emerge even from heavily infested brood cells.

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Table I. Body weight of *A. mellifera* drone pupae slightly or moderately infested with *V. destructor* mites. The meanings of abbreviations are: SD = standard deviation, r = range, w.l. = % weight loss, p = *t*-test probability.

Pupal eye color	Uninfested brood cells (without mites)	Slightly infested brood cells (with 1–3 female mites)	Moderately infested brood cells (with 4–6 female mites)
White	$\bar{x}$ = 342.5 mg SD = 26.7 mg r = 249–413.4 mg *n* = 177	$\bar{x}$ = 345.5 mg SD = 18.4 mg r = 291–390.2 mg *n* = 75 w.l. = 0%	$\bar{x}$ = 339.4 mg SD = 16.9 mg r = 310–360.7 mg *n* = 9 w.l. = 0%
Pink	$\bar{x}$ = 326.9 mg SD = 31.7 mg r = 235.7–409.5 mg *n* = 187	$\bar{x}$ = 328.5 mg SD = 31.7 mg r = 240.3–403.8 mg *n* = 151 w.l. = 0%	$\bar{x}$ = 335.3 mg SD = 20.6 mg r = 311.1–361.8 mg *n* = 8 w.l. = 0%
Red	$\bar{x}$ = 318.0 mg SD = 32.2 mg r = 246.8–400.4 mg *n* = 266	$\bar{x}$ = 313.3 mg SD = 30.3 mg r = 218.8–394.4 mg *n* = 396 w.l = 1.5% *P* = 0.15	$\bar{x}$ = 307.3 mg SD = 23.9 mg r = 225.2–353.6 mg *n* = 46 w.l. = 2.5% *P* = 0.04
Brown	$\bar{x}$ = 288.5 mg SD = 20.6 mg r = 230.9–386.7 mg *n* = 191	$\bar{x}$ = 275.6 mg SD = 29.2 mg r = 189.3–345 mg *n* = 238 w.l. = 3.8% *P* < 0.0001	$\bar{x}$ = 271.9 mg SD = 29.1 mg r = 216.4–325.8 mg *n* = 51 w.l. = 4.6% *P* < 0.0001
Emerging adults	$\bar{x}$ = **277.1 mg** SD = 16.0 mg r = 235.2–376.1 mg *n* = 158	$\bar{x}$ = **245.2 mg** SD = 28.0 mg r = 171.9–320.5 mg *n* = 119 w.l. = 11.3% *P* < 0.0001	$\bar{x}$ = **223.9 mg** SD = 33.5 mg r = 144.8–226 mg *n* = 89 w.l. = 18.3% *P* < 0.0001

2. MATERIALS AND METHODS

In spring and summer of 1999, we collected combs with capped drone brood from colonies derived from *A. mellifera carnica*, kept in the apiaries of the University of Tübingen. We transferred the frames into an incubator, opened brood cells and removed pupae with white, pink, red and brown eyes. In addition, we sampled emerging adult drones from the same combs. Because we removed these drones from their brood cells, their wings were not fully developed so that malformations could not be classified with certainty. We determined the number per cell of adult female mites, distinguished by their darker cuticle and signs of wear, and weighed the host bees on a microbalance (Kern 770) to the nearest 0.1 mg. Data from 2 235 cells were used for the calculation of weight loss. In order to compare our results with those of previous work, especially Schneider and Drescher (1987), we compared the reduction in weight for groups of slightly (1–3 mites) and moderately (4–6 mites) infested pupae. We used *t*-tests (Sall and Lehman, 1996) to evaluate the significance of different mean values.

3. RESULTS

Uninfested white eyed drone pupae weighed on average 342.5 mg and newly emerged adults 277.1 mg (Tab. I). Infested brood cells contained up to 20 adult female mites with most cells containing 1–5 mites. We recorded a reduced weight in emerging drones even if only one female mite was present in a brood cell. They weighed on average 250.4 mg (standard deviation 30.1 mg) which is 10% less than uninfested controls.

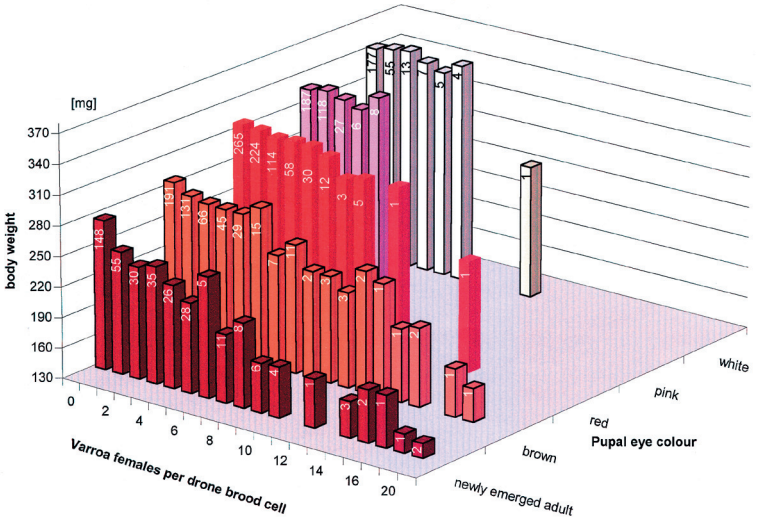

Figure 1. Weight of *Apis mellifera* drones during pupal development in relation to multiple infestation of brood cells by *Varroa destructor* mites. Bars = means, numbers on bars = n brood cells. The weight loss progresses especially during late pupal stages. Even some heavily infested individuals were capable to emerge.

Weight loss increased with multiple infestation of an individual brood cell (Fig. 1), especially from the red eyed pupal stage on. The few drones that emerged from cells infested by up to 20 adult female mites and their offspring reached only half the normal drone body weight (Fig. 1).

In comparing the effects of drone brood cell invasion by 1–3 and 4–6 female mites, we found in both groups a significant weight loss from the brown-eyed stage onwards, and a considerably reduced body weight in the emerging adults (Tab. I).

4. DISCUSSION

Our data on weight loss in individual drones of the western honey bee, *A. mellifera*, in relation to the parasitic load caused by brood infestation by *V. destructor* mites revealed two yet unknown facts: first, drones emerge with significantly diminished weight even if only one female mite has invaded the brood cell. We suppose that the fitness of these males will be reduced. Though sperm production and viability of drones slightly infested by mites during preimaginal development were recently described as normal (Collins and Pettis, 2001), their participation in reproduction remains questionable (Rinderer et al., 1999; Sylvester et al., 1999). Second, a few mini-drones were capable of emerging even if 20 female mites with progeny were found in their brood cells. In worker brood, 4–6 female mites per cell prevent host emergence in most cases (De Jong et al., 1982). The high percentage of drones reaching the adult stage despite increased levels of pupal infestation by *V. destructor,* as documented here for *A. mellifera,* is in sharp contrast to the situation in the original host bee of these mites, the Asian species *Apis cerana* Fabr. (Rath, 1992). In *A. mellifera* colonies, part of the heavily infested pupae is removed by the adult worker bees (Aumeier, 2001) which does not occur in *A. cerana* colonies (Boecking, 1999), resulting in the trapping and death of many female mites.

The weight loss related to the level of mite infestation became evident especially during the late pupal phase when, in addition to the adult female mites, their progeny was also sucking haemolymph from the host. In comparison to the data of Schneider and Drescher (1987) on reduced weight of adult drones as a consequence of a slight or moderate degree of pupal infection, the weight loss recorded in our sample of emerging males was considerably greater. Studies on more parameters

presumably influencing the fitness of individual drones are currently being carried out.

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Résumé – Perte de poids des nymphes mâles d'*Apis mellifera* suite à une infestation multiple par l'acarien *Varroa destructor*. Les pertes de colonies d'abeilles domestiques (*Apis mellifera* L.) sont principalement dues aux lésions causées aux ouvrières par l'acarien *Varroa destructor* au cours de leur développement imaginal. Il existe là-dessus une abondante littérature, mais on connaît comparativement peu de choses sur les lésions causées aux mâles. C'est pourquoi nous avons étudié chez 2 235 nymphes mâles et mâles naissants, la perte de poids provoquée au cours de la phase préimaginale par un parasitisme plus ou moins fort. Nous avons ouvert des cellules de couvain operculé, dénombré les parasites et compté le nombre d'acariens femelles adultes par cellules. Nous en avons trouvé jusqu'à 20, mais la plupart des cellules infestées renfermaient de 1 à 5 acariens femelles. Nous avons ensuite pesé les abeilles avec une précision de 0,1 mg. Les mâles naissants non parasités pesaient en moyenne 277,1 mg. Lorsqu'il n'y avait qu'une seule femelle d'acarien par cellule, la perte de poids des mâles adultes était déjà d'environ 10 %. La perte de poids augmentait parallèlement à l'infestation (Fig. 1). Des abeilles adultes arrivaient encore à émerger des cellules qui renfermaient 15 à 20 femelles d'acariens et leur descendance, mais il s'agissait alors de mini-mâles qui ne pesaient que la moitié des mâles témoins non parasités. La perte de poids des individus parasités survenait principalement vers la fin de la phase nymphale ; elle était significative aussi bien chez les nymphes aux yeux marrons que chez les adultes naissants (Tab. I). C'est la première fois que sont décrits de façon quantitative les effets du degré de parasitisme des nymphes mâles sur le poids des adultes. Les résultats montrent qu'une infestation préimaginale par *Varroa destructor* atteint les mâles par une perte de poids et que chez *Apis mellifera* des mini-mâles peuvent émerger des cellules fortement parasitées. Ceci est à l'opposé de ce qui se passe chez l'hôte d'origine, *Apis cerana*. Des études en cours portent sur les facteurs supposés influencer la fitness des mâles à poids réduit.

Varroa destructor* / infestation multiple / couvain de mâles / perte de poids imaginale / fitness des mâles / *Apis mellifera

Zusammenfassung – Gewichtsverlust bei mehrfach von Milben (*Varroa destructor*) parasitierten Drohnenpuppen (*Apis mellifera*). Verluste von Völkern der Honigbiene gehen wesentlich auf individuelle Schädigungen von Arbeiterinnen zurück, die durch Parasitierung während der Puppenphase entstehen. Hierüber existiert eine umfangreiche Literatur, jedoch ist vergleichsweise nur wenig über entsprechende Effekte bei Drohnen bekannt. Wir untersuchten daher an insgesamt 2 235 Puppen und gerade schlüpfenden adulten Drohnen, welcher Gewichtsverlust durch unterschiedlich starke Parasitierung in der präimaginalen Phase verursacht wird. Dazu wurden verdeckelte Brutzellen geöffnet, auf Milbenbefall kontrolliert und gezählt, wieviele erwachsene Milbenweibchen eingedrungen waren. Wir fanden bis zu 20, die meisten der befallenen Zellen enthielten jedoch 1–5 *Varroa*-Weibchen. Dann wurden die Bienen auf 0,1 mg genau gewogen. Nicht parasitierte, schlüpfende Drohnen wogen durchschnittlich 277,1 mg. Wenn in die Brutzelle nur ein Milbenweibchen eingedrungen war, betrug der Gewichtsverlust der adulten Drohnen bereits etwa 10 %. Mit zunehmendem Befallsgrad wurde ein immer stärkeres Mindergewicht registriert (Abb. 1). Es schlüpften erstaunlicher Weise auch aus einigen der 15–20 Milbenweibchen und deren Nachkommen enthaltenden Zellen noch erwachsene Bienen, allerdings waren dies Minidrohnen, die nur halb so schwer waren wie die unparasitierten Kontrollen. Der Gewichtsverlust befallener Individuen trat vor allem gegen Ende der Puppenphase auf, er war bei braunäugigen Puppen ebenso wie bei schlüpfenden Imagines signifikant (Tab. I). Die hier erstmals quantitativ beschriebenen Effekte bestimmter Parasitierungsgrade von Drohnenpuppen auf das Adultgewicht zeigen, daß ein präimaginaler Befall durch Milben von *Varroa destructor* stets eine durch Gewichtsverlust erke-nnbare Schädigung bewirkt, und daß bei *Apis mellifera* sogar aus stark befallenen Brutzellen Drohnen schlüpfen können, wenn auch nur Minidrohnen. Dies steht im Gegensatz zu den Befunden bei der ursprünglichen Wirtsart *Apis cerana*. Wie die Fitness mindergewichtiger *mellifera*-Drohnen zu beurteilen ist, soll in weiterführenden Untersuchungen geklärt werden.

Varroatose / Mehrfachparasitierung von Drohnenbrut / präimaginaler Gewichtsverlust / Männchen-Fitness / *Apis mellifera* / *Varroa destructor*

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