

## Number and position of wounds on honey bee (*Apis mellifera*) pupae infested with a single *Varroa* mite

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**Abstract.** The wounds inflicted on pupae in capped brood cells of the honey bee, *Apis mellifera*, infested with a single female of the ectoparasitic mite, *Varroa destructor*, were investigated after visualisation by vital staining with trypan blue. On average the mites made two integumental perforations for feeding on prepupae and one on pupae. Most of the punctures were on particular ventral sites on the abdomen. Possible reasons for this pronounced preference and the evolutionary aspects of this highly specialised parasite-host relationship are discussed.

### INTRODUCTION

The mite *Varroa destructor* (Anderson & Trueman, 2000) is a haemolymph-sucking ectoparasite of the Asian honey bee, *Apis cerana* (Rath, 1999). Due to the intercontinental bee trade, this mite was transferred to a new host *Apis mellifera* and is now distributed worldwide, causing severe problems as it damages honey bee colonies (Morse & Nowogrodzki, 1999). Reproduction of the mite is restricted to capped brood cells (Ifantidis & Rosenkranz, 1988), which are invaded by female mites a few hours prior to their being sealed (Aumeier et al., 2002). These mites puncture the integument of last instar bee larvae, prepupae and pupae with their chelicerae and the perforations are repeatedly used by the *Varroa* female and her offspring to feed on haemolymph of the host (Donzé & Guerin, 1994).

Because these processes occur within capped brood cells, transparent artificial brood cells were developed to observe this parasitism (Abbas & Engels, 1990; Donzé & Guerin, 1994). However, the integumental wounds on the host bee are difficult to detect. Therefore, their number and exact position could not be studied in detail. This became possible by using vital staining of the wounds with trypan blue (Kanbar & Engels, 2004). Here we present data on the position and number of cuticular punctures on preimaginal host bees infested by a single female mite. The results are discussed under aspects of evolutionary adaptations in this parasite-host relationship.

### MATERIAL AND METHODS

We used heavily infested colonies of *Apis mellifera carnica* in the apiaries of the University of Tübingen. In the experimental hives, which were not treated with *Varroa* control measures in spring and early summer, many brood cells contained *Varroa destructor* mites. Combs with capped brood were collected and stored in an incubator at 35°C and 80% humidity. More than 2,000 sealed worker and drone brood cells were carefully opened and inspected for the presence of mites. From about 1,100 cells, which only one female mite had invaded, the host bee was removed and incubated at room temperature for 30 min in a ringer medium containing 0.01% trypan blue (Chroma, Stuttgart, # 1 F 577). By this procedure epidermal cells damaged (compare Roche Lexikon Medizin, 1999, p. 1602) by mite-made punctures, became vital-stained (Kanbar & Engels, 2004). The

integumental wounds of the host bee appeared as small blue spots. They were studied under a photo-stereomicroscope (Wild M 8) at up to 80 × magnification.

### RESULTS

#### Appearance of trypan blue stained wounds

The trypan blue staining of integumental perforations on late L5 feeding instars was unsuccessful. But the wounds of prepupal stages onwards were successfully vital stained and could be easily detected as small blue dots. Shortly after the pupal moult, the central hole of these perforations measured about 50–60 µm in diameter (Fig. 1a). Because the mites repeatedly use this feeding site, more surrounding epidermal cells become damaged and the wound enlarges in the course of the pupal phase (Fig. 1b). In brownish pigmented pupae (Fig. 1c) the perforations may reach a diameter of 80–100 µm, or more.

#### Frequency of punctures on host bees

The percentage of host individuals with *Varroa*-made perforations, visualised by the trypan blue staining, increased in the course of preimaginal development (Fig. 2). Over 3/4 of all infested prepupae had integumental wounds. In later pupal stages wounds were found on nearly all bees. In the drone brood there was always a slightly higher fraction of individuals with punctures than in the worker brood.

#### Position of the wounds on the host bee

There were no punctures on the anterior region, comprising head, thorax and the first abdominal segment, or the last abdominal segments of prepupae (Fig. 3). Perforations were found on abdominal segments 2 to 9, mostly on the left ventral side. About 40% of all wounds were on the 6<sup>th</sup> abdominal segment of both worker and drone pupae.

On pupae a high percentage of the wounds was on the 2<sup>nd</sup> abdominal segment, indicating that the female mite had a different preferred feeding site on pupae. Very few perforations were found on segments 3 to 5 of the abdomen (Fig. 4). Again nearly all the punctures were on the sternites, of worker as well as of drone pupae, and 60% were positioned more towards the left side. Surprisingly, on about 1/4 of the worker pupae the wounds were located on the mesothorax (Fig. 4). We want to emphasise that no males in the over 1,100 host bees investigated

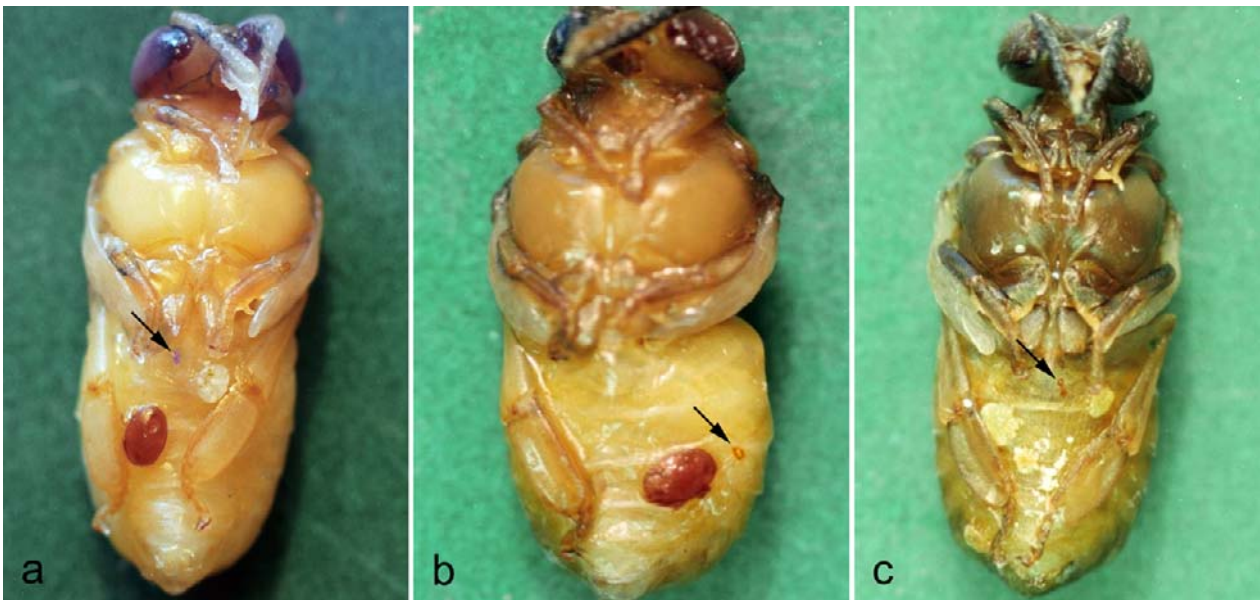


Fig. 1. Honey bee (*Apis mellifera*) drone pupae in ventral view, vital stained with trypan blue to visualise integumental wounds (arrows) made by *Varroa* females for sucking host haemolymph. (a) Pupa about 16 days old with one perforation on the 2<sup>nd</sup> abdominal segment, (b) pupa about 18 days old with one perforation on the 4<sup>th</sup> abdominal segment, (c) pupa about 20 days old with two perforations on the 2<sup>nd</sup> abdominal segment. Brown adult *Varroa* females are seen in a and b, pale nymphal mites in a and c.

for this study and the one thousand or more drone pupae inspected for other reasons in our laboratory over the last five years had *Varroa*-made perforations on their thorax.

#### Number of wounds per host bee

The number of punctures on late feeding and spinning L5 bee larvae could not be determined with certainty. On prepupae we counted one to three perforations, with on average two. There was no difference in the number of wounds between worker and drone individuals. On the majority of pupae there was only one wound present. Two perforations (Fig. 1c) were found on 1.7% and 2.7% of about 600 pupae of workers and 600 of drones, respectively. Three wounds were detected on only one worker and one drone pupa.

## DISCUSSION

#### Number of punctures

There are few records in the literature on the number of perforations made by *Varroa* females as permanent feeding sites on capped honey bee brood, especially in cells infested by a single

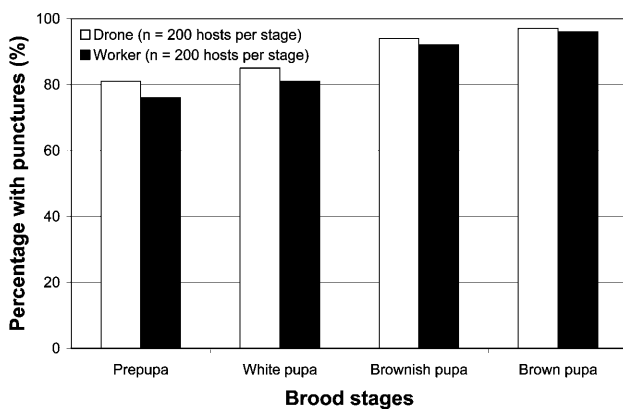


Fig. 2. Percentage of preimaginal brood stages of the honey bee (*Apis mellifera*) on which *Varroa*-made integumental wounds were detected by vital staining with trypan blue.

mite. There are on average two on prepupae and one on pupae removed from normal wax cells of combs in naturally infested colonies showing severe symptoms of varroaosis. Donzé & Guerin (1994) mentioned higher numbers, but they used artificial transparent and round brood cells for their video recordings. Nevertheless, they observed more wounds on prepupae than pupae. The fact that we detected only one perforation on nearly all the worker and drone pupae we examined may be because the female mite prepares this feeding site for long-term use. Immediately after the pupal moult the *Varroa* female pushes the hindlegs of the bee host aside and punctures an abdominal sternite (Donzé & Guerin, 1997). According to these authors, this is performed quickly, probably because sclerotisation of the cuticle makes puncturing of the cuticle more difficult. Another question is how many punctures are present on brood infested by more than one mite. Up to 20 *Varroa* females may invade a drone brood cell (Duay et al., 2002).

#### Position of the wounds

Most of the mite inflicted wounds on prepupae were on the 6<sup>th</sup> and on pupae on the 2<sup>nd</sup> abdominal sternite. The latter confirms the video records of Donzé & Guerin (1994) who, how-

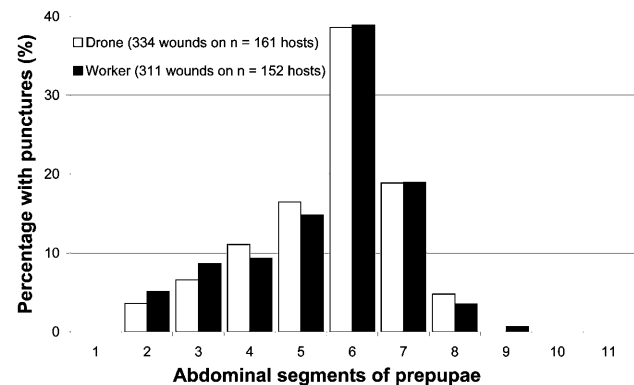


Fig. 3. Percentage of abdominal segments of prepupal host bees (*Apis mellifera*) with *Varroa*-made integumental wounds.

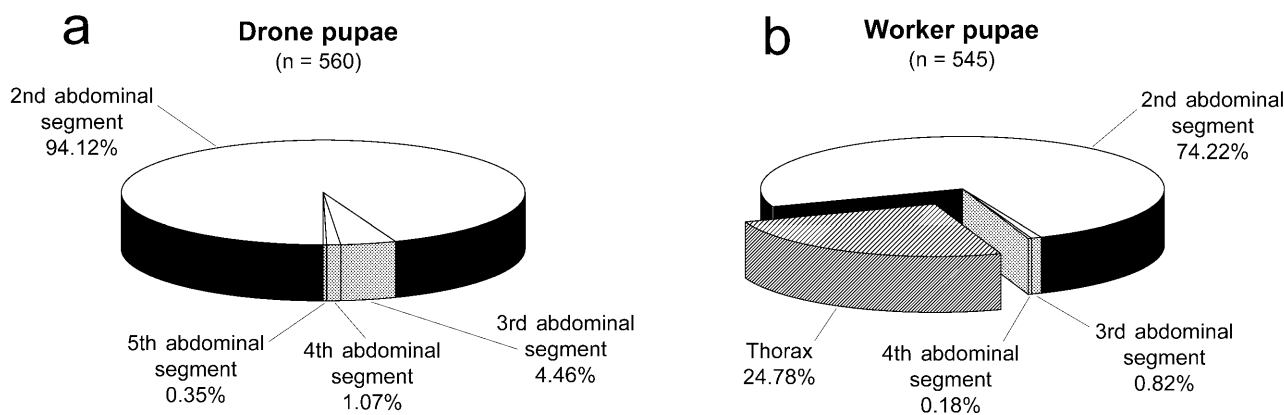


Fig. 4. Pie diagram of the percentage of *Varroa*-made integumental wounds on various parts of the body of bee pupae (*Apis mellifera*). The 2<sup>nd</sup> abdominal segment is the preferred site on drone (a) and worker pupae (b) but perforations on the thorax *only* occurred on worker pupae.

ever, named this abdominal segment 5 because they included the three thoracic segments. Such an exact positioning of the feeding site on the host's body is probably a result of selection. Unfortunately, nothing is known about the wounds made by *Varroa* females feeding on the brood of its original host species, *Apis cerana* (Rath, 1999). We assume that the position of the perforations on *A. mellifera* prepupae and pupae provides optimum for haemolymph uptake by the mites.

#### Mite orientation towards the site of the puncture

*Varroa* mites can only reproduce in capped brood cell of honey bees. There is limited space, but the conditions are absolutely stable: a wax-lined narrow cell, high humidity and constant temperature of about 35°C. The only source of nutrition for the female mite, and later on her progeny, is one preimaginal host bee. In order to feed on the bee's haemolymph, the body wall of the host has to be perforated. It is not surprising that the evolution of this very specific parasite-host relationship resulted in the mite selecting a particular feeding site, which is located with great accuracy. *Varroa* females are capable of accurately locating and puncturing the bee's integument at a few specific sites, presumably optimum for haemolymph uptake.

What determines these preferred sites, and how does the parasite recognise them? The 2<sup>nd</sup> abdominal sternite of pupae in particular is chosen with remarkable constancy (Fig. 4). Interestingly, preferential sucking of haemolymph from this segment in other host insects is also known for related taxa, like mussel mites (Baker, 1991). This raises questions about the special properties of this feeding site. In honey bee pupae the sternite of the 2<sup>nd</sup> abdominal segment is somewhat stretched over an underlying large haemocoelic cavity. We attempted to puncture the ventral region of the abdomen with a needle and found it was particularly easy on the 2<sup>nd</sup> sternite. The tendency for the abdominal punctures on pupae to be mostly a little left of the midline suggests an additional and unknown orientation stimulus. More *Varroa* mites were also found on the left side of the abdomen of adult bees (Delfinado-Baker et al., 1992).

#### Worker brood infestation in *Apis mellifera*

In colonies of *A. mellifera* the parasite-host relationship is evidently still unbalanced (Rosenkranz, 1999). If suffering from varroatosis, they are often co-infected with virus (Martin, 1998). In the original host, the Asian *A. cerana*, female *Varroa* mites invade worker brood but reproduce exclusively in drone brood (Koeniger et al., 1981; Tewarson et al., 1982; Boot et al., 1997). Why in *A. mellifera* colonies *Varroa* mites are also able to reproduce in worker brood is unknown (Garrido et al., 2001). As

shown here, only on worker pupae are 1/4 of the *Varroa*-caused wounds found on the thorax (Fig. 4).

#### Ultimate consequences of the injury to bee pupae

The limited number of wounds made by *Varroa* mites may be an adaptive trait, because these perforations in the pupal cuticle are routes for microbial infection (Brødsgaard et al., 2000), which will eventually kill the bee, because only towards the end of the pupal phase will a scar form over the wounds (Kanbar & Engels, 2003). If the adult bee does not emerge from its cell, the mites are trapped in the sealed brood cell, as is observed in heavily infested drone brood of *A. cerana* (Boecking, 1999). Limited exploitation and minimum damage to the host will therefore increase the fitness of the *Varroa* female as it does not adversely affect the mite's reproductive potential (Boot et al., 1997). Such factors may have shaped the behaviour of this successful honey bee parasite.

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