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## Regular dorsal dimples on *Varroa destructor* – Damage symptoms or developmental origin?\*

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**Abstract** – Adult females ( $n = 518$ ) of *Varroa destructor* from *Apis mellifera* prepupae were examined by scanning electron microscopy without prior fluid fixation, dehydration and critical-point drying. Fifty-five (10.6%) mites had one (8.1%) or two (2.5%) diagonal dimples positioned symmetrically on the idiosoma's dorsum. Where one such regular dorsal dimple existed per mite body, it occurred on the left or right side, equally. Mites with at least one regular dorsal dimple were 3.4% longer, but neither idiosomal width nor elliptical area differed significantly from mites lacking dimples. Dimple length was normally distributed in the population, and averaged  $462 \pm 9.2 \mu\text{m}$  (s.e.;  $n = 68$  dimples). Internally, each regular dorsal dimple aligns with a series of obliquely-situated, dorso-ventral muscles in the opisthosoma. It is concluded that regular dorsal dimples are faults originating during mite ontogeny and should be considered separately from damage to *Varroa destructor* inflicted by honeybees or predatory arthropods.

**developmental fault / idiosoma / regular dorsal dimple / scanning electron microscopy / *Varroa destructor***

### 1. INTRODUCTION

Since spreading to colonies of the European honey bee (*Apis mellifera* L.), much interest has been directed at identifying colonies that actively defend themselves against infestation by parasitic mites of *Varroa* spp. (reviewed by Boecking and Spivak, 1999). Commonly these mites are groomed and bitten by worker bees of their natural host species, *A. cerana* Fabricius (Peng et al., 1987; Büchler et al., 1992; Fries et al., 1996), a behaviour also exhibited in certain colonies of *A. mellifera* (reviewed by Boecking and Spivak, 1999; Corrêa-Marques et al., 2000; Stanimirović et al., 2005).

To identify honey bee colonies that injure *Varroa destructor*, mites fallen to the bottom board frequently are sorted to characterize individuals potentially damaged by a

hive's worker bees. The procedure has associated risks, in that naturally-fallen mites which die may subsequently be damaged by house-cleaning workers (Rosenkranz et al., 1997; Bienefeld et al., 1999) or may be injured or go missing owing to activities of a hive's non-bee inhabitants (Szabo and Walker, 1995; Lodesani et al., 1996; Thakur et al., 1997; Bienefeld et al., 1999). Moreover, it is questionable whether all irregularities on the mite body should be regarded as handling damage from arthropods. To be able to accurately quantify the frequency and understand the types of injuries received by *V. destructor*, it is essential for surveys of mite morphology to carefully discriminate damage symptoms from deformities of a developmental nature.

Upon the idiosoma of fallen mites of *V. destructor*, many studies have distinguished dorsal indentations from other body abnormalities (Morse et al., 1991; Ruttner and Hänel, 1992; Lodesani et al., 1996; Rosenkranz et al., 1997; Bienefeld et al., 1999; Harbo and Harris,

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1999a, b; Corrêa-Marques et al., 2000, 2002; Zaitoun et al., 2001; Stanimirović et al., 2005). But few have questioned whether these types of body imperfections may arise during mite ontogeny (Lodesani et al., 1996; Rosenkranz et al., 1997). The “single or double hollow on dorsal shield (Figs. 2, 4a–c)” and in Table II, “hollow of the dorsal shield” were identified as types of damage, but later discussed as possible “accidental events in the growth stages of the nymphs or during the sclerotization process” (Lodesani et al., 1996). Rosenkranz et al. (1997) reported “regular dimples on the dorsal shield” of living mites and attributed them to “disturbances during the nymphal development”, citing the previous paper. Related descriptions, recognized as mite damage, have also been given. Morse et al. (1991) found dead mites with “one or two indentations in the dorsal surface” and attributed these to worker bee mandibles. Some young adult females had “dents on their idiosoma” (Harbo and Harris, 1999a) or “dents in the dorsal surface of the idiosoma” (Harbo and Harris, 1999b) upon cell departure as progeny; these depressions were reported to have been impossibly caused by the mandibles of grooming bees but rather, possibly from leg movements of the host bee while inside the brood cell (Harbo and Harris, 1999a). Moreover, a “depression (hollow) in the dorsal shield” (Tab. 3 of Corrêa-Marques et al., 2000), an “indentation or hollow in the dorsal shield” (Tab. 2 of Corrêa-Marques et al., 2002), and “depression or hollow in the dorsal shield” (Tab. 2 of Stanimirović et al., 2005) have been identified, but the accompanying illustrations (Figs. 2, 4 of Corrêa-Marques et al., 2000; Fig. 4-B5 of Stanimirović et al., 2005) show features different from those displayed by Lodesani et al. (1996).

While investigating the impact of different ant species as natural agents causing mortality of *V. destructor* in the laboratory (Davis et al., 2007a), a morphological abnormality of occasional incidence but predictable location and certainly distinct from damage symptoms caused by ant mandibles and chemical sprays (Davis et al., 2007b; in preparation), was discovered in mites whether exposed to ants or not. Accordingly, the purpose of this study was to address the standing question of inflicted

damage by arthropods versus faults in *V. destructor* development, to specifically address the following objectives: to (i) quantify the frequency of one and two regular dimples, and their length, on the dorsum of the mite idiosoma; (ii) compare the size of mites possessing regular dorsal dimples with those lacking dimples; (iii) relate the precise location of regular dorsal dimples to the mite’s internal structure; and (iv) resolve confusion from the literature by providing clear images and a standardization of terminology for reference.

## 2. MATERIALS AND METHODS

### 2.1. Source and treatment of mites

Most mites of *Varroa destructor* (Anderson and Trueman, 2000) examined in this study were those from earlier experiments (Davis et al., 2007a). Briefly, mites were collected during May–July 2005 from brood frames of two queenright colonies of hybrid Italian honey bees (*Apis mellifera ligustica* Spinola) from the same apiary (N52° 03.741' W106° 38.704'). The colonies had not been treated chemically in any way.

Mites were harvested immediately prior to experimentation. Because immature drones were infrequent, more than 85% of mites came from sealed worker cells, from multiple frames. All mites there were dark brown adult females (prior to oviposition) which adhered to the visually-healthy, motionless prepupae (neither mature larvae nor pupae) when withdrawn using forceps. From the prepupae, individual mites latching onto a new child’s paint brush or forceps were gently transferred until ten mites had been introduced to each 60 mm petri-dish arena lacking ants (controls) or holding five ants; trials were performed separately for each of three species (*Formica fusca* group, *Lasius neoniger*, *Tapinoma sessile*). Sealed arenas, not supplied with additional food or water, were held in darkness at 24 °C. Arenas either allowed contact between the five ants and ten mites, or only their vapours to pass. Once sealed, mites and ants remained in arenas until death of all arthropods. Trials were replicated five to usually seven times and lasted up to 132 h.

Additional to the original study (Davis et al., 2007a), ten mites harvested in exactly the same way before placement into contact arenas lacking ants, were instead immediately frozen. Four trials were performed.

## 2.2. Examination of mite morphology

With the intent to document how published injuries to mites of *V. destructor* inflicted by worker honey bees might vary from those due to ants (Davis et al., in preparation), all mites were gently gathered from arenas. Each of the 518 dead mites was observed preliminarily using a stereomicroscope (40 $\times$ ) to detect macroscopic irregularities before examination by scanning electron microscopy (SEM). Without any of the conventional steps of fluid fixation, dehydration or critical-point drying, naturally air-dried individuals simply were mounted in appropriate orientation on double-sided tape adhering to labeled aluminum stubs. Stubs containing mites were stored in closed specimen boxes protected from dust. After gold coating (Edwards sputter coater S150B), mites were examined at 30 kV using a Philips 505 SEM and photographed using Polaroid 665 P/N film, or high-density printing paper (UPP-110HD Type II) on a Sony UP-860 Video Graphic Printer.

From these images (approx. 70 $\times$ ) of mites having regular dorsal dimples, maximum width (not including the lateral-margin setae; Figs. 1–4) and length of the idiosoma were measured, allowing elliptical area ( $\pi \times \frac{1}{2}$  max. width  $\times$   $\frac{1}{2}$  max. length) to be calculated per mite. For comparison, 75 mites lacking dimples were selected evenly from, but randomly within, the three treatments [i.e., 25 mites each from arenas where they were separated from ants, from arenas that allowed contact by ants, and from arenas without ants (control)]. Using one-tailed t tests ( $\alpha = 0.05$ ), idiosomal dimensions were compared between mites with and without regular dorsal dimples.

Although dimple depth was difficult to quantify accurately, maximum length of each regular dorsal dimple was measured from these photographs and the means calculated and compared among these three treatments (t tests,  $\alpha = 0.05$ ).

## 3. RESULTS

### 3.1. Occurrence, frequency and variable length of regular dorsal dimples

Ordinarily, the dorsal surface of the idiosoma of mature adult female mites of *V. destructor* is convex and unblemished, clothed in pilose setae of fairly uniform distribution but increasing length toward the shield's posterior

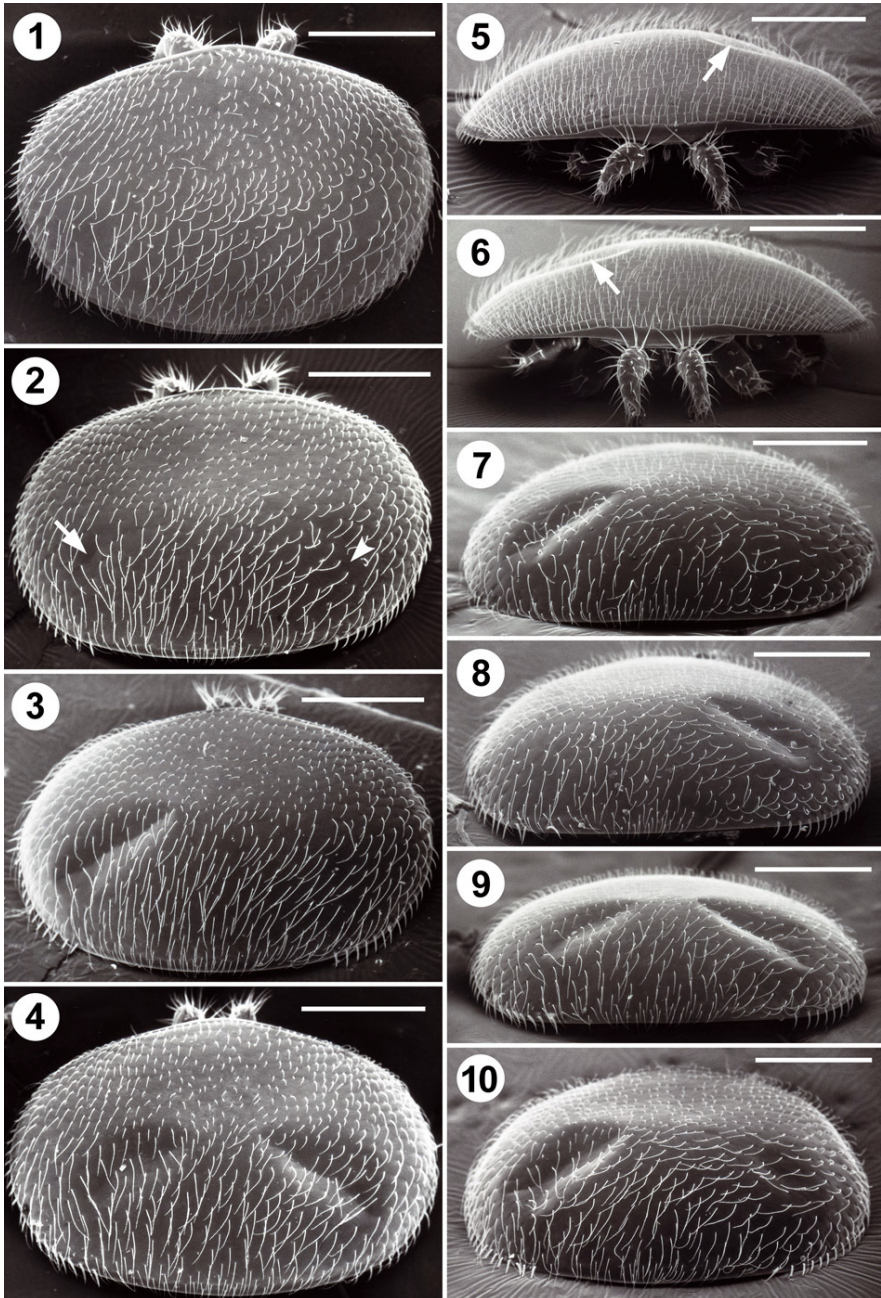
(Fig. 1). However, in 8.7–12.9% of mites from various treatments, including 9.8% of those without any exposure to ants (i.e., control) (Tab. I), the idiosoma possessed one (Fig. 3) or a maximum of two (Fig. 4) depressions on the dorsum in a regular location. Overall, 10.6% (55) of the 518 total mites examined had dorsal dimples; 8.1% (42 of 518) had a single, and 2.5% (13 of 518) had double dimples, respectively (Tab. I).

When viewed dorsally, such dimples were slanted toward the idiosoma's midline of maximum length, almost always without merging, typically yielding a bilaterally-symmetrical appearance in a high proportion of that minority of mites (23.6%; 13 of 55) which possessed two dimples (Figs. 4, 9; Tab. I). Especially by tilting to view in posterior-dorso perspective, however, it was evident that the degree of dimpling could vary on the same mite (Fig. 10).

Mean lengths of the regular dorsal dimples were not significantly different ( $P > 0.3$ ) among the three treatments (contact by ants, separation from ants, absence of ants). Thus, dimple lengths were combined and overall, they were normally distributed (Fig. 11) around the mean of  $462 \pm 9.2$  (s.e.,  $n = 68$  dimples; range 287–660  $\mu\text{m}$ ). On the 13 mites with double dimples, dimple lengths differed on average by 8.6%. Five mites had extraordinary matches in dimple length (i.e., only 0.3 to 1.0% difference; Fig. 4), whereas dimple lengths on the same mite varied up to 28% (Fig. 10).

On certain mites that either lacked dimples entirely or had only the slightest of dents, regions on the idiosoma corresponding to locations where dimples might once have been forming, were evident (Fig. 2).

Forty-two of the 55 mites (76.4%) had just one dorsal dimple, rather than two, still in a regular position. With 20 and 22 mites possessing only a left (Figs. 3, 5, 7) or right (Figs. 6, 8) dorsal dimple, respectively (Tab. I), these solitary depressions were distributed equally ( $\chi^2_{1df} = 0.0952$ ;  $P > 0.75$ ). Therefore, when all 68 regular dorsal dimples encountered in this study are considered, the distribution of 33 left and 35 right dimples did not deviate statistically from a 1:1 ratio ( $\chi^2_{1df} = 0.0588$ ;  $P > 0.8$ ).



**Figures 1–10.** Scanning electron micrographs of mature adult female mites of *Varroa destructor*. **Figs. 1–4.** Dorsal view of idiosoma. (1) Dimples absent. (2) Very short and shallow dimple (arrow) at left. Lack of dimple in corresponding position that is relatively bare (arrowhead), at right. (3) Left dimple alone. (4) Left and right dimples (double dimple). **Figs. 5–6.** Anterior view. (5) Left dimple alone (arrow). (6) Right dimple alone (arrow). **Figs. 7–9.** Posterior view of idiosoma. (7) Left dimple alone. (8) Right dimple alone. (9) Same mite as Figure 4, showing two dimples. (10) Dorsal-posterior view of mite with two dimples; right dimple shallower and shorter than left. Scale bars = 0.5 mm.

**Table I.** Frequency of one or two regular dorsal dimples on the idiosoma of adult female mites of *Varroa destructor* taken from sealed cells containing honeybee prepupae. Following collection, mites lacked any exposure to ants (control) or were enclosed in petri-dish arenas either separated from, or in direct contact with, ants (L = left dimple alone; R = right dimple alone).

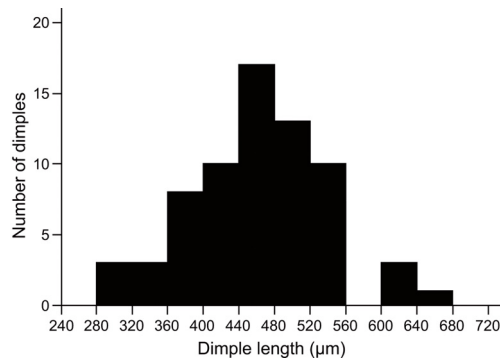
Treatment	No. mites examined	No. mites with regular dorsal dimple(s)			Total dimples
		Total (%)	Single dimple	Double dimple	
<u>CONTROL</u>					
Immediately frozen	33	3 (9.1)	2 (1L, 1R)	1	4
In arenas without ants	69	7 (10.1)	5 (1L, 4R)	2	9
<i>Control Total</i>	102	10 (9.8)	7 (2L, 5R)	3	13
<u>EXPOSURE TO ANTS</u>					
In arenas separated from ants	210	27 (12.9)	23 (13L, 10R)	4	31
In arenas contacted by ants	206	18 (8.7)	12 (5L, 7R)	6	24
<b>Total</b>	<b>518</b>	<b>55 (10.6)</b>	<b>42 (20L, 22R)</b>	<b>13</b>	<b>68</b>

From the total of 55 mites with regular dorsal dimples, the ratio of 20:22:13 (mites with a left dimple alone: mites with a right dimple alone : double dimpled mites) accorded to a 2:2:1 distribution in a goodness-of-fit test ( $\chi^2_{2df} = 0.273$ ;  $P > 0.85$ ).

Also, to investigate whether single regular dorsal dimples could have resulted as damage from handling by mandibles of honeybees or other arthropods, checks of the ventral idiosoma were made to search for a corresponding indentation or mutilation marks. Only when a regular dorsal dimple was accentuated, was it possible to detect a concavity at the junction of the metapodal and genital plates (Fernandez et al., 2006), on that same side of the mite in a location ventrally opposite the dimple. Thus, externally, most regular dimples were detectable only dorsally, without any obvious sign ventrally.

### 3.2. Dimensions of mites with and without regular dorsal dimples

Each mite encountered with dorsal dimple(s) ( $n = 55$ ) was measured and mean dimensions compared to 75 associated mites lacking dimples. When mites were assigned to categories of none, one, at least one, or two



**Figure 11.** Frequency distribution of the maximum lengths of 68 regular dorsal dimples on 55 adult female mites of *Varroa destructor* taken from cells containing prepupae of *Apis mellifera*.

dimples, no difference in idiosomal width was registered (Tab. II). However, a statistically significant ( $P < 0.01$ ) increase in maximum idiosomal length was revealed in all mites with dimples compared to those with dimples absent (Tab. II). Mean body-length increases ranged from 2.3% (one dimple) to 3.4% (double dimples). However, these incremental increases in length of dimpled mites did not result in statistically greater elliptical areas of the idiosoma (Tab. II).

**Table II.** Dimensions (mean  $\pm$  s.e.) of the idiosoma of adult female mites of *Varroa destructor* with zero, one, at least one, or two regular dorsal dimples. Means in columns with different letters as superscript are significantly different ( $\alpha = 0.05$ ).

No. regular dimples	No. mites examined	Maximum width (mm)	Maximum length (mm)	Elliptical area (mm <sup>2</sup> )
0	75	1.488 <sup>a</sup> $\pm$ 0.009	1.009 <sup>a</sup> $\pm$ 0.004	1.180 <sup>a</sup> $\pm$ 0.011
1	42	1.478 <sup>a</sup> $\pm$ 0.015	1.033 <sup>b</sup> $\pm$ 0.008	1.201 <sup>a</sup> $\pm$ 0.019
2	13	1.483 <sup>a</sup> $\pm$ 0.020	1.044 <sup>b</sup> $\pm$ 0.012	1.217 <sup>a</sup> $\pm$ 0.029
1 + 2	55	1.479 <sup>a</sup> $\pm$ 0.012	1.035 <sup>b</sup> $\pm$ 0.007	1.205 <sup>a</sup> $\pm$ 0.016

## 4. DISCUSSION

Results from this study plus published data from several others lead to the conclusion that some mites of *Varroa destructor* develop one or two depressions in predictable locations on the dorsum of the idiosoma. Proposed terminology and a discussion about their occurrence and possible formation, follow.

### 4.1. Terminology

Throughout this article and recommended for future reference, the term “regular dorsal dimple” is used to represent each of up to two depressions per mite that occur in positions on the idiosoma illustrated in Figure 4. This usage (i) is preferred over “one or two indentations in the dorsal surface” (Morse et al., 1991) and “single or double hollow in the dorsal shield” (Lodesani et al., 1996) which initially had been used to categorize types of damage and are terms now established in the literature to mean variable symptoms of damage clearly associated with injuries caused by honeybees (see Introduction); and (ii) is consistent with “regular dimples on the dorsal shield” stated to denote a developmental disturbance (Rosenkranz et al., 1997) dissociated from arthropod-inflicted injury.

### 4.2. Genetic evidence favouring a developmental origin of regular dorsal dimples

Although the following two studies have reported idiosomal abnormalities of *Varroa* females as “damage”, their results nonetheless

provide significant genetic evidence, directly or indirectly, that such irregularities could have arisen during mite development. Of the damage reported as “single or double hollows on dorsal shield (Figs. 2, 4a–c)” by Lodesani et al. (1996), evidently the first and second figures illustrate regular dorsal dimple(s) on the mite idiosoma. Lodesani et al. found that from worker brood cells, 17.9% of the female mites that were light-coloured, first-offspring mature progeny had damage to the idiosomal cuticle if their mother also had dorsal-shield damage, compared to 2.8% when the foundress lacked idiosomal faults.

Harbo and Harris (1999a) recorded different types of “physical damage to mites” among bee colonies. They found “dents on the idiosoma” of young adult female mites not to be caused by adult bees, these progeny mites possessing dents before they left honeybee brood cells for the first time. Unlike mite injuries designated “broken legs or bodies”, the heritability ( $h^2$ ) of these “dents in body” among colonies was  $0.00 \pm 0.45$ , and was concluded to provide an inferior prospect for selective breeding. Another interpretation of the zero heritability value is that it may have resulted because regular dorsal dimples are a phenomenon of mite development, rather than damage inflicted by honeybees, and so would be predicted not to vary, nor be heritable, among bee colonies.

Results from these important studies, when viewed from an alternative perspective, are consistent with these idiosomal irregularities occurring during mite ontogeny, and with the potential for defects like regular dorsal dimples to be passed on to the next mite generation without handling by honeybees.

### 4.3. Formation of regular dorsal dimples – a developmental perspective

In the adult female population of *V. destructor* studied here, 10.6% of mites on honeybee prepupae had at least one regular dorsal dimple. Interestingly, Lodesani et al. (1996) found 10.2% of mites entering honeybee brood cells had “dorsal shield damage”. Here, the fraction of left to right dimples equaled 1:1, and the ratio of mites with left dimple alone : right dimple alone : two dimples, approximated 2:2:1. Assuming no difference in survival and brood-cell entry between mites with one versus two regular dorsal dimples, these proportions are very consistent with expectations of an occasional event during mite ontogeny, unbiased to the left or right position and without an obligation to occur together, such that a prediction of four mites having a single regular dorsal dimple for every mite having the maximum of two dimples, has been met.

Although the existence of two regular dorsal dimples per mite (i.e., double dimple) on honeybee prepupae was a relatively rare condition – a frequency of just 2.5% overall – it is important in that it shows a bilateral symmetry of the event. The location of regular dorsal dimples was highly precise, and hence predictable.

Moreover, the presence of small quantities of modified larval jelly (confirmed by its pollen content; Haydak, 1970; Matsuka et al., 1973) within some regular dorsal dimples (data not shown), presumably acquired during entrapment dorsally (Ifantidis, 1988; Martin, 1994), demonstrates that these depressions existed on these foundresses prior to brood-cell entry. Thus, being already present in this study when mites were harvested from cells of prepupae, the dimples themselves were not injuries caused later by ants in the laboratory trials (Davis et al., unpublished). Indeed, compared to other treatments (9.1–12.9%) including controls without ants, regular dorsal dimples were recorded least often (8.7%) in mites contacted by ants (Tab. 1).

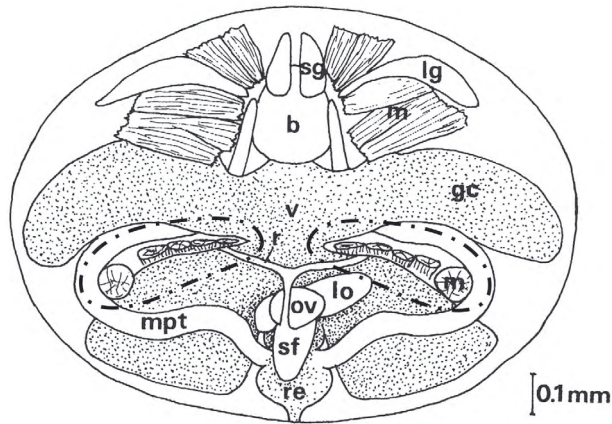
Anatomically, the regular dorsal dimples observable on the fully-sclerotized adult female mite have a corresponding alignment,

in the same oblique orientation, to two series of five muscles within the idiosoma (Fig. 12; de Ruijter and Kaas, 1983). These diagonally-situated muscles, which may be the “dorsal adducent muscles” referred to by Ionescu-Varo and Suci (1979), are attached dorso-ventrally between the middle and posterior caecal lobes of the ventriculus, located on each side internally to the wide-sweeping loop of each of the mite’s Malpighian tubules (Fig. 12). Indeed, on general musculature in the Acarina, Baker and Wharton (1952) wrote “The origins and insertions of muscles can be detected externally because they frequently produce visible scars on the plates and dimple-like concavities in the softer integument”.

The following arguments are conjectural, but may assist future investigation of the formation of regular dorsal dimples in *V. destructor*. It is hypothesized that the incidence of these idiosomal defects is manifested by natural selection on *V. destructor* for rapid progeny development within the sealed honeybee brood cell, a temporary habitat of opportunity (Walter and Proctor, 1999). In honeybee parasitic mesostigmatan mites such as *V. destructor* and *Tropilaeleps clareae*, which will provide a useful comparison, suppression of an intervening stage of development, the tritonymph, means that the deutonymph moults directly into an adult (Delfinado-Baker et al., 1985; Steiner, 1995; Walter and Proctor, 1999). In *T. clareae*, for which idiosomal irregularities equivalent to *V. destructor*’s regular dorsal dimples have not been found in the literature, the deutonymph of variable but unspecified size (Delfinado-Baker et al., 1985) increases to  $976 \times 528 \mu\text{m}$  (length  $\times$  width) in the adult female (Delfinado and Baker, 1961). Unlike *Varroa*, the oblong body with elongate legs in adults of both sexes, is not adapted for creeping beneath an adult bee’s abdominal segments; instead, *T. clareae* is more general in its distribution on the host bee (Rath et al., 1991), often only nestling between the body domains (head-thorax; thorax-abdomen) as shown in *A. dorsata* (Büchler et al., 1992; Delfinado-Baker et al., 1992) and *A. mellifera* (Ritter and Schneider-Ritter, 1988).

In *V. destructor*, on the contrary, the direct ontogenetic leap from female deutonymph to





**Figure 12.** Line drawing of internal structure of adult female *Varroa* mite showing obliquely-oriented muscles (m) situated within large invaginations between the middle and posterior gastric caecal lobes (gc) on each side of the midgut. These muscles, which align with the position of regular dorsal dimples that form occasionally and are evident on the idiosomal surface, are encircled by dotted lines which approximate the dimple contours. b – brain; lg – lateral gland; lo – lyrate organ; mpt – malpighian tubule; ov – ovary; r – ramus; re – rectum; sf – sacculus foemineus; sg – salivary gland. The drawing, originally published by A.A. Balkema, Rotterdam, has been modified slightly from Figure 1 of de Ruijter and Kaas (1983), and is reproduced here with permission of the Office for Official Publications of the European Communities.

adult requires a large increase in body width to attain the flattened, crab-like shape enabling entry between the abdominal segments of adult honeybees of *A. cerana* and *A. mellifera* (Hänel, 1988; Delfinado-Baker et al., 1992), to reduce losses to host grooming. Indeed, female idiosomal width in *V. destructor* increases from 485  $\mu\text{m}$  in the protonymph to 750–800  $\mu\text{m}$  in the deutonymph (reviewed by Fernandez et al., 2006), before a dramatic doubling in width to between 1.5 mm (Tab. II) and 1.7 mm (Anderson and Trueman, 2000), in the adult.

The circulatory system in Acarina is lacunary and consists only of blood (Baker and Wharton, 1952; Ionescu-Varo and Suci, 1979) and intramoult increases in body size presumably rely on hydrostatic pressure to bring about the necessary swelling or unfolding of previously-deposited cuticle lying within the current developmental life stage (Walter and Proctor, 1999). *V. destructor*'s ultimate body width packaged within the deutonymph's framework demands a major, one-stage expansion of the new surface cuticle to reach its elliptical body shape before sclerotization becomes prohibitive. It is postulated

that hydrostatic pressure within the immature mite's adult body is occasionally insufficient to fully inflate the idiosoma's large dorsal cuticle prior to its hardening, leading to these depressions becoming permanent in up to two regular locations per mite.

Lagging of final idiosomal expansion at the dimple regions may be a common phenomenon in the female's deutonymph-to-adult transition, and the frequency of mites with double dimples would seem to rise if not for the expansions at potential dimple locations to occur independently to some extent. That is, failure at full inflation at only one side of the dorsal shield was 3.2-fold more frequent than failure to fully expand at both. Independence of full expansion at the two possible dimple regions could also account for the population's impartiality of left and right dimples among mites with only one. This view is favoured over the possibility of a solitary indentation in a regular dorsal dimple location to have occurred by way of injury inflicted by arthropod (e.g., honeybee, ant) handling, which potentially could happen coincidentally. But if force sufficient to cause a regular dorsal dimple had resulted from closure of arthropod

mandibles, notwithstanding a potentially increased armour due to multiple sclerotized plates on the mite's ventral surface, it seems reasonable to expect some sign of corresponding mutilation damage caused by the other mandible on the ventral idiosomal surface or legs, that corresponded to the regular dorsal dimple location, opposite. However, no such ventral damage was detected in mites with regular dorsal dimple(s).

It is possible that constraints in lateral body expansion of female *V. destructor* brought about during formation of one or two regular dorsal dimples, might increase hydrostatic pressure internally in the mite's antero-posterior plane, resulting in a compensatory, barely elongated body in mites with dimple(s) compared to those without.

Overshadowing the frequency of one or two regular dorsal dimples and the predictability of their location is the clear gradation in their final length on fully-sclerotized adults, regardless of any subsequent exposure to ants. This continuum in dimple length, too, favours a gradual idiosomal expansion prior to sclerotization, resulting in phenotypes where dimples are just barely detectable to deep, and even show distinct differences in concavity on the same individual. Again, instances like the latter also suggest a positional independence of cuticle expansion by the same mite.

Some connection between placement of the regular dorsal dimples and the diagonally-arranged muscles circled in Figure 12 seems assured, but careful developmental studies are required to determine whether factors such as muscle reduction or defect in development, or malfunctions such as prolonged contraction or the inability to relax, might be involved. Determination of exactly how the musculature below regular dorsal dimples is involved will shed further insight into the apparent combination of concurrent physiological events, involving a gradual cuticle expansion on the one hand and the advance of protective sclerotization on the other, which in some mites eventually precedes full inflation and makes the developmental fault permanent.

Though not a form of damage, events leading to regular dorsal dimple formation in female mites occur naturally in progeny only,

and hence within the sealed honeybee brood cell (Lodesani et al., 1996; Harbo and Harris, 1999a), prior to sclerotization of the mite body being complete. Although an astute suggestion and possible to have occurred coincidentally in rare instances, particularly where only one regular dorsal dimple is present, it seems highly improbable that leg movements by the host pupa or emerging adult bee (Harbo and Harris, 1999a) could imprint the soft idiosoma of a maturing daughter mite twice, dorsally, in precisely the bilaterally-symmetrical pattern observed with regularity in this study. Indeed, it would be interesting to determine whether a large central dent of non-symmetrical contour on the dorsal idiosoma, similar to Figure 2 of Corrêa-Marques et al. (2000), could result from a lucky blow by the tip of one of the host's legs while inside the brood cell.

#### 4.4. Long-term consequences for mites with regular dorsal dimples

While much is to be learned about the life history and fate of *V. destructor* mites with one or two regular dorsal dimples, the literature already may contain pertinent information. For example, if the "single or double hollow on dorsal shield" of Lodesani et al. (1996) equates to the regular dorsal dimples of this study, the slight difference in adult body length determined here seems not to interfere with the mite's ability to creep between the adult bee's abdominal segments, allowing access to hemolymph, a reduced loss to grooming, and potentially to be carried away to another colony, by the bee. No adult female mites having damaged legs were found to be phoretic on worker honeybees, but 7.2% of phoronts did have a "hollow of the dorsal shield", prompting the suggestion of an "apparent irrelevance of dorsal shield damage", for the mite (Lodesani et al., 1996). In addition, the frequency of mites with dorsal-shield irregularities only, that fell to hive bottom boards, was not significant (Lodesani et al., 1996). Furthermore, a mite with at least one regular dorsal dimple still entered a brood cell, fed on the immature host, and apparently reproduced (Fig. 2 of Lodesani et al., 1996), despite its

idiosomal defect. However, overall, there may be a detrimental effect on numbers of daughter mites produced, and hence on mite population growth within a colony, because 35.7% of dorsal-shield damaged mites yielded no offspring, compared to just 14.5% of foundress mites which were undamaged (Lodesani et al., 1996). Because the mites of that study possessed more idiosomal irregularities (based in Figs. 4a–c) than just regular dorsal dimples, it would be useful to study reproductive fitness of mites whose only idiosomal faults were regular dorsal dimples.

## 5. CONCLUSIONS AND RECOMMENDATIONS

Results of this study suggest that regular dorsal dimples arise as developmental faults during mite ontogeny. Thus, when surveying mites of *V. destructor* mites for signs of body damage, there is a need to establish separate categories for those mites possessing regular dorsal dimple(s), excluding them from mites damaged by arthropods. However, it should be noted that mites which had developed with regular dorsal dimples definitely can still receive idiosomal damage thereafter, at least from ants (Davis et al., unpubl. data).

Several basic and applied aspects about regular dorsal dimples on *V. destructor* remain to be investigated, including their actual cause and process of formation during mite ontogeny. To this end, it would be informative to extend studies to other *Varroa* species, to determine whether occurrence and size of regular dorsal dimples correlate with descending body width: *V. destructor*, *V. rindereri*, *V. jacobsoni* and *V. underwoodi* (Anderson and Trueman, 2000).

Furthermore, there was evidence that mite occurrence and multiplication can occur within the regular dorsal dimples (data not shown), possibly connected to the routine but temporary entrapment of the foundress mite (Ifantidis, 1988; Pugh et al., 1992; Martin, 1994; Harbo and Harris, 1999b) dorsally in residual larval food of the honeybee host. Accordingly, it would be useful to survey the type and fate of the contents of regular dorsal

dimples of foundress and progeny mites, upon natural departure from brood cells at host eclosion.

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**Les dépressions dorsales régulières sur *Varroa destructor* : marques de blessures ou irrégularités dans l'ontogenèse ?**

***Varroa destructor* / morphologie / ride dorsale / développement post embryonnaire / défaut de développement / microscopie électronique à balayage**

**Zusammenfassung – Regelmäßige Grübchen auf dem Rücken von *Varroa destructor* – Zeichen von Verletzungen oder Entwicklungsstörungen?** Um vom Normalen abweichende Merkmale am Körper von *Varroa destructor* zutreffend auf durch Honigbienen oder andere Arthropoden hervorgerufene Verletzungen zurückführen zu können, ist es wichtig, diese von durch Unregelmäßigkeiten in der Entwicklung von *V. destructor* hervorgerufene Symptome unterscheiden zu können. Von Mai bis Juli 2005 wurden adulte Weibchen (n = 518) von *V. destructor* aus Zellen mit Vorpuppen von *Apis mellifera* (zumeist Arbeiterinnenzellen) eingefroren oder in Laborversuchen in Gruppen von 10 in Petrischalen über bis zu 132 Stunden bis zu ihrem Tod gehalten (Kontrollen), oder Ameisen ausgesetzt (entweder mit Kontakt oder dem Geruch allein). Natürlicherweise in Luft getrocknete Milben wurden mit Rasterelektronenmikroskopie (SEM) untersucht, ohne die üblichen Prozeduren wie Flüssigkeitsfixierung, Entwässerung mit Lösungsmitteln oder einer Kritische Punkt Trocknung. SEM der Rückenseite des Idiosoma (Abb. 1) zeigten, dass 55 (10,6 %) der Milben eine (8,1 %) oder zwei (2,5 %) diagonale Grübchen in symmetrischen Positionen aufwiesen (Abb. 2–10; Tab. I). Nur bei Grübchen von beträchtlicher Tiefe war auch auf der Bauchseite der Milben eine Einsenkung an der Verbindung der metapodialen Platten und der Genitalplatten vorhanden, die mit der Position der dorsalen Einsenkungen korrespondierte.

Für diese Einsenkungen, die in der Literatur als von den Mandibeln oder Beinritten der Bienen hervorgerufene Verletzungen angesehen werden, wird empfohlen, sie zukünftig als "reguläre dorsale Grübchen" zu bezeichnen. Diese regulären dorsalen Grübchen variierten in Tiefe und Ausdehnung (Abb. 2 und 3), sogar innerhalb der gleichen Milbe (Abb. 10). Die maximale Länge der regulären dorsalen Grübchen lag im Durchschnitt bei  $462 \pm 9,2 \mu\text{m}$  (Standardfehler;  $n = 68$ ) Grübchen und war normalverteilt (Abb. 11). Bei Milben mit nur einem regulären dorsalen Grübchen hatten 20 das Grübchen auf der linken Seite (Abb. 3, 5 und 7), und 22 hatten sie auf der rechten Seite (Abb. 6, 8; Tab. I). Bei 55 Milben mit regulären dorsalen Grübchen entsprach das Verhältnis von 20 Milben mit einem Grübchen nur auf der linken Seite und 22 mit einem Grübchen nur auf der rechten Seite sowie 13 Milben mit Grübchen auf beiden Seiten in einem Anpassungstest einem Verhältnis von 2:2:1 ( $\chi^2_{2df} = 0,273$ ;  $P > 0,85$ ). Milben mit zumindest einem regulären dorsalen Grübchen waren im Schnitt bis zu 3,4 % länger, allerdings unterschied sich weder die Breite des Idiosomas noch die elliptische Fläche signifikant von der von Milben ohne Grübchen (Tab. II). Im Inneren der Milben liegt jedes der regulären dorsalen Grübchen in einer Linie mit einer Reihe von schrägergerichteten an Rücken und Bauchseite befestigten Muskeln an jeder Seite des Mitteldarms der Milben (Abb. 12). Ein Versagen bei der Umwandlung der weiblichen Deutonymphe zu einem ausgewachsenen Tier von dem Doppelten der früheren Breite des Idiosoma vor der endgültigen Sklerotisierung voll Aufzublähen führt offensichtlich zur beständigen Ausbildung der dorsalen Vertiefungen in einer mit diesen Muskeln des Opisthosomas übereinstimmenden Ausrichtung. Es wird geschlossen, dass die regulären dorsalen Grübchen Entwicklungsstörungen darstellen, die während der Ontogenie der Milben entstehen und von den durch Arthropoden verursachten Verletzungen von *V. destructor* getrennt gesehen werden müssen. Weitere Untersuchungen sind nötig, um die Ereignisse bei der Ausbildung der Grübchen sowie irgendwelche Konsequenzen für die Milben weiter zu klären.

#### Entwicklungsstörungen / Idiosoma / reguläre dorsale Grübchen / Rasterelektronenmikroskopie / *Varroa destructor*

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