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THE EMBRYONIC DEVELOPMENT OF VARROA JACOBSONI
OUDEMANS 1904 (VARROINAE, MESOSTIGMATA)

BY Dietrich MAUTZ *, Werner HIRSCHMANN ** and Franz KEMNITZER ***

ABSTRACT: for the first time the authors document the existence of different embryonic stages of Varroa jacobsoni OUDEMANS 1904 the honey bees mite. The outstanding peculiarity of Varroa's development is demonstrated by the fact, that primarily a sixlegged, and later an eightlegged immobile embryo is formed, from which finally a mobile protonymph originates. A mobile and free living sixlegged larva does not occur, and this contrasts to the development in related mite species.


INTRODUCTION

KANG-CHEN and ROU-SU (1975) based their description on one egg and three embryos of Varroa jacobsoni and in their abstract they conclude: "The female gets into cell just before it is capped and lays dormant six-legged larvae on the bee pupa or cell wall every day or two days. In the first the egg forms in the ovary, and it develops an embryo in the uterus until become a complete larva, and then deposited". In fig. 5 a broad oval egg is illustrated with six legs at its front part. Fig. 6 (cit. "Dormant six-legged larve of V. jacobsoni just deposited ") demonstrates the egg shape of the rear body part while at the front body part the two palps as well as the six strong legs are visible. The original small figures (1,5 × 1,0 cm) do not show any other detail or setae of the embryo's body or legs. The body dimensions of embryos described are given by 620 × 510 μ (fig. 5-6 in KANG-CHEN a. ROU-SU, 1975, p. 52).

While researchers in many parts of the world are anxiously searching for control methods of the bee mite Varroa, no further morphological studies on the embryonic stages of the parasite have come...
to the authors' knowledge. The intention of this investigation is an accurate description of the embryonic stages, with a particular attention placed on any sixlegged stage of the mite.

**MATERIALS AND METHODS**

Different developmental stages of *Varroa jacobsoni* were obtained from drone brood cells of *Apis mellifera carnica* Pollmann colonies. On 5th of June, 1985, brood combs were collected for this purpose from a *Varroa* infested apiary in Lower Frankonia, West-Germany (Bavaria, Johannishof near Lengfurth a.M.).

After carefully uncapping the drone cells and removing the drone pupae (in the white eyed stage) we could get eggs and embryonic stages along with fertile mite females. By naked eye we could see translucent to opaque white tiny bubbles, and small whitish mites with legs. With a fine sable paint brush we transferred all small white stages to collecting glass vessels filled with fixative (ethanol 70% : 90 ml; acetic acid 99% : 5 ml; formaldehyde 40% : 5 ml).

The soft stages were carefully flattened under close scrutiny below a stereomicroscope (SR, Zeiss) and embedded in polyvinyl lactophenol.

Microphotos were taken with a Zeiss III photomicroscope using the “DIK” procedure after NOMARSKI (Zeiss S. 41-210.Z.5-d, may 11, 1975), with the aid of phase contrast technic or a polarised light applyance (c.f. legends). The magnification of the micrographs is indicated on each figure.

**RESULTS: DESCRIPTION OF THE IMMOBILE DEVELOPING STAGES OF *VARROA JACOBSONI***

*FIGURE 1*: Egg of *Varroa*; dimension 656 × 596 μ (short and long axis respectively).

1.1 *polarised light micrograph*

The egg is broadly oval, its oblong shape is well perceptible; the egg appears transparent, hollow, anisotropic eggsubstances are decernable as whitish grains or clouds.

1.2 *DIK micrograph*

Focus on egg surface: The egg shell and vesiculated yolk substance is clearly visible. The main yolk mass appears shifted downwards and retreated from the egg envelope; folds across on the right half of the egg are assumed preparation artefacts; no germination nor any step towards the development of an embryo are discernible.

*FIGURE 2*: Early sixlegged embryonic stage within the egg with primary development of muscle fibres; lateral view; dimension : 698 × 545 μ.

2.1 *DIK micrograph*

Six tenuous legs of the embryo are clearly visible at one end of the egg; the embryo seems to fill about 20% of the egg, and the yolk substance the rest.

2.2 *polarised light micrograph*

As in fig. 1.1 anisotropic egg substances appear as whitish grains or marginal clouds, they are in greater number than in fig. 1.1. The area of the embryo (upper left) remains free of anisotropic substance. In the upper right of the egg developing cross-striated muscles appear.

This developmental stage is comparable the one given by KANG-CHEN and ROU-SU (1975, fig. 5).

*FIGURE 3*: Further advanced sixlegged embryonic stage within the egg with well defined muscle system; dorsal view; dimension : 647 × 536 μ.

3.1 *DIK micrograph*

The further advanced embryo with palps and six legs is distinctly bulging the egg shell; legs
and palps are bent to venter and consist of greater volume than those in picture 2.1; at the front part of the dorsal side some very short, needle-like dorsal setae are noticeable; at the rear part of the egg round as well as laterally broadly oval vesicles can be observed between the egg shell and yolk masses; about one half of the egg is filled by the embryo.

3.2 polarised light micrograph
Compared with picture 2.2 fewer anisotropic substances can be recognized as white grains or marginal white vesicles; the inner front part is provided with cross-striated muscles. Diagonally passing striated bands look like propable leg muscles.

3.3 DIK micrograph, high magnification (oil immersion)
Two dorsal setae are shown in high magnification; setae are acute conical with concave base.

3.4 DIK micrograph, high magnification (oil immersion)
Three leg setae are illustrated in high magnification; the setae are of acute conical shape with concave base but notably shorter than dorsal setae.

The same embryo examined by phase contrast technique slightly shows the development of a fourth legpair.

This developmental stage is corresponding to fig. 6 of KANG-CHEN a. ROU-SU (1975).

FIGURE 4: Eightlegged embryonic stage with primary development of protonymphal peritrema; ventral view; dimension: 698 × 535 μ.

4.1 DIK micrograph
The picture shows a stepwise further advanced immobile embryonic stage. Its legs and palps are bent to the inside, leg III is pointing forwards, leg IV towards the rear inside. The embryo seems to fill 2/3ds of the egg. The yolk masses appear retreated from the egg shell at the back and laterally, thus causing a small light cavity band.

4.2 phase contrast micrograph
The picture documents the primary development of the protonymphal peritrema within this embryonic stage; recognize the separate chambers of the peritrema; other organs of the protonymph could not be recognized.

FIGURE 5: Eightlegged embryonic stage with starting development of protonymphal legs; ventral view; dimension: 664 × 494 μ.

5.1 DIK micrograph
At the rear part vesicles are formed between the folded, translucent egg shell and embryo; the palps and legs are distinctly pointing diagonally towards the inside. At the front margin the egg shell can still be recognized.

5.2 phase contrast micrograph
The palps and legs I, II, III are illustrated in magnified section; within the embryonic legs the protonymphal legs are forming. At the apical part of leg III two short cone shaped setae of the embryo are visible and, between them one longer seta of the protonymphal leg. At the front between right leg I and palps one ventral seta can be recognized.

FIGURE 6: Eightlegged embryonic stage within partially burst egg shell including a further developed protonymph; lateral view; dimension: 732 × 562 μ.

6.1 DIK micrograph
In the front half the embryonic legs with setae are visible, the legs are bent against each other. At the front part of the egg the shell is burst, its remainder can be noticed in upper right. At
Fig. 1: egg of *Varroa jacobsoni*. 1.1. - Polarised light micrograph. 1.2. - DIK micrograph, scale = 250 µm.

Fig. 2: early sixlegged embryonic stage, lateral view. 2.1. - DIK micrograph (L₁₋₃ = legs), scale = 250 µm. 2.2. - Polarized light micrograph (M = muscle).

Fig. 3: further advanced sixlegged embryo, dorsal view. 3.1 - DIK micrograph (L₁₋₃ = legs, P = palps, DS = dorsal setae), scale = 250 µm. 3.2. - Polarised light micrograph (M = muscle). 3.3. - DIK micrograph, oil immersion (DS = dorsal setae), scale = 25 µm. 3.4. - DIK micrograph, oil immersion (LS = leg setae), scale = 25 µm.

Fig. 4: eightlegged embryonic stage with protonymphal peritremata, ventral view. 4.1. - DIK micrograph (L₁₋₄ = legs, P = palps), scale = 250 µm.
the rear part the translucent egg shell is completely present. No more yolk masses are visible.

6.2 polarised light micrograph
Anisotropic substances are visible as white grains or marginal vesicles. Cross-striated muscles are spread over the whole embryo, in present focus they are particularly visible within the right body half, where leg muscles are inserted.

6.3 phase contrast micrograph
By higher magnification the peritrema of the protonymph can be recognized which is fully developed; the peritrema is of voluminous, racemose shape, with more than 20 oval separate chambers (c.f. illustration of peritrema after HIRSCHMANN 1980, p. 62, fig. 2).

6.4 phase contrast micrograph
The leg setae are illustrated by magnified section; the short, cone shaped leg setae of the embryo with concave base look darker than the setae underneath belonging to the protonymph, which are long, spearshaped. The protonymphal setae seem to be not yet fully developed.

FIGURE 7: Fully developed eightlegged protonymph. Egg shell burst to its greatest extent; no egg yolk visible, shape of this stage still egg like, oblong oval; legs and palps still bent inwards, thus immobile. Dimensions: 638 × 494 µ.

7.1 DIK micrograph
Focus on dorsal surface: in the rear left of the stage its translucent egg shell is still maintained. The polytrichous dorsal setae appear notably clear at the rear part of the body. In the centre of the dorsal surface a crossfold is existing. Directly in front of this crossfold a dorsal setae-free area should be noticed (c.f. fig. 17 in HIRSCHMANN 1980, p. 64). The spearlike, strong dorsal setae of the rear body part are pointing to the rear, they are inserted in small knobs.

7.2 DIK micrograph
Focus on ventral surface: the hairy bent palps and legs are difficult to recognize, since the preparation is focused on the ventral setae and anal area. Between insertion of legs three pairs of sternal setae are visible. At the rear part of the body about 20 ventral setae are situated in front of the skepshaped anus. The anale is of oblong oval shape including the cribrum. Ventral setae are somewhat shorter than dorsal setae but provided with distinct small insertion knobs as well. At the rear body marginal setae are visible, which are still rising within the egg shell. The marginal setae are partially pointing to the front. Circumanal setae are pointing to the anus, while other ventral setae pointing to diverse direction (c.f. fig. 14 in HIRSCHMANN 1980, p. 63: ventral surface of protonymph).

7.3 phase contrast micrograph
Rear margin and anal area in magnified section: the marginal setae within egg shell are bent to a hook; circumanal setae are acute and slightly bent. The cribrum is provided with 7 to 8 crossrows of very tiny dents pointing towards the front. Within one crossrow about 30 to 40 of those dents can be counted.

FIGURE 8: Eightlegged, free, mobile protonymph. In contrast to fig. 7 body shape not oblong oval, but almost circular; palps and legs radially stretched off from body. Dimension: 527 × 487 µ.

8.1 DIK micrograph
Ventral view: at the front the stretched cheliceres are visible between palps, beyond the cheliceres gnathosoma with corniculi, and, between coxae 1 tritosternum. The ventral setae and anal area are similar to those illustrated in fig. 7.2. The marginal setae of the rear body are radially standing off, and are not bent nor even pointing to the front as in pictures 7.1 and 7.2. The setae of leg coxae are visible better on left than on right.
4.2. - Phase contrast micrograph (PE = Protonymphal peritrema), scale = 50 µm.

5.1. - DIK micrograph (L₁₋₄ = legs, P = palps), scale = 250 µm. 5.2. - Phase contrast micrograph (GN = gnathosoma, L₁₋₃ = legs, LAS = Larval seta, P = palp, PRS = protonymphal seta, VS = ventral seta), scale = 100 µm.

6.1. - DIK micrograph (L₁₋₄ = legs, P = palps), scale = 250 µm. 6.2. - Polarized light micrograph (M = muscles). 6.3. - Phase contrast micrograph (PE = protonymphal peritrema), scale = 50 µm. 6.4. - Phase contrast micrograph (LAS = larval seta, PRS protonymphal seta of legs), scale = 50 µm.

7.1. - DIK micrograph, dorsal view (DS = dorsal seta, MAS = marginal seta), scale = 250 µm. 7.2. - DIK micrograph, ventral view (A = anale, LS = leg seta, L₁₋₄ = legs), scale = 250 µm.
FIG. 7 (continuation): fully developed eight-legged protonymph, legs and palps still bent inwards. 7.3. — Phase contrast micrograph, anal area (A = anale, AS = anal seta, CR = cribrum, MAS = marginal seta), scale 100 μm.

FIG. 8: eight-legged, free, mobile protonymph. 8.1. — DIK micrograph, ventral view (A = Anale, CO = corniculi, CH = cheliceres, GN = gnathosoma, L₁₄ = legs, LS = leg seta, MAS = marginal seta, P = palp, VS = ventral seta), scale = 250 μm.

8.2. — DIK micrograph dor:>'nal view (GN = gnathosoma, DS = dorsal seta, L₁₄ = legs, LS = leg seta, MAS = marginal seta, P = palp), scale = 250 μm.
8.2 *DIK* micrograph

Dorsal view: the polytrich dorsal setae are similar to those of fig. 7.1; marginal setae of the rear body part are protruding radially. They are distinctly visible better than in fig. 8.1, as well as dorsal setae of legs.

**DISCUSSION**

The embryonic development of *Varroa jacobsoni* Oudemans 1904 is documented by a series of 21 microfotos. The development starts with an undifferentiated egg (fig. 1) and runs through a sixlegged (fig. 2 and 3), eightlegged (fig. 4 to 6) embryonic stage, through a still immobile but fully developed protonymph, the egg shell of which is partially present (fig. 7), finally to the free mobile protonymph (fig. 8).

Since we could not define the exact age of the different stages, we are not able to determine the duration of development of these stages. Furthermore we must admit, that the exact dimensions of the different stages may differ from our estimation (c.f. materials and methods: artificially flattening of the stages). The question keeps open whether female varroa mites exclusively deposit undifferentiated eggs (which we could demonstrate) or those which are in embryonic development or fully developed protonymphs as stated by KANG-CHEN and ROU-SU (1975).

Within the gamasid family a free living, sixlegged larva originates by suppression of legpair IV during embryonic development *. This sixlegged larva seems not to occur within *Varroa* development, as so far it has not been found. Our results demonstrate this: within the egg there is developing a sixlegged, later an eightlegged embryo, in which the final eightlegged protonymph is forming. From the view of our results the definition egg larva or prelarva seems not correct. It is not even a dormant larva (KANG-CHEN and ROU-SU, 1975, c.f. above), since the sixlegged stage still is in embryonic development. By this peculiarity the honey bee parasite *Varroa* has been able to shorten its period of development. And more than that, the protonymph with its distinct polytrich setae has gained higher resistance than a hypothetical tender sixlegged larva with less setae.

**LITERATURE CITED**


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