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Ontogenesis of *Varroa jacobsoni* Oud

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SUMMARY - This review article deals with characteristics of ontogenesis of the honey bee ectoparasitic mite *Varroa jacobsoni* Oud. Research data include timing of ontogenesis, sex ratio, morphological differentiation between males and females, order of descendants in relation to the time they are deposited as eggs in the sealed brood, stages and duration of development, number of depositing eggs, output of mature daughters per reproductive cycle, feeding behaviour and, finally, mortality of developing mites. Data presented here have been obtained from the early 80's and are the result of research carried out mainly on European races of *Apis mellifera* L.

Key words: *Varroa jacobsoni*, egg laying periodicity, developing stages, family composition, sexual dimorphism, juvenile mortality, *Apis mellifera*.

RESUME - *"Ontogenèse de* Varroa jacobsoni *Oud." Cet article porte sur les caractéristiques de l'ontogenèse de l'acarien ectoparasite de l'abeille mellifère,* Varroa jacobsoni *Oud. Les données de la recherche ont rapport au moment de l'ontogenèse, au ratio sexuel, à la différenciation morphologique entre femelles et mâles, à l'ordre de la descendance selon le moment où ils sont pondus comme oeufs dans le couvain scellé, étapes et durée du développement, nombre d'oeufs pondus, production de filles matures par cycle reproductif, comportement alimentaire, et finalement, mortalité des acariens en développement. Les données présentées ici ont été obtenues à partir des années 80 et sont le résultat de recherches faites principalement sur des races européennes de* Apis mellifera *L.*

Mots-clés *:* Varroa jacobsoni*, périodicité de la ponte, étapes de développement, composition de la famille, dimorphisme sexuel, mortalité des jeunes,* Apis mellifera*.*

Introduction

Ontogenesis (from the Greek on, ontos = "being", and genesis = "creation") is the development of a living organism from egg to sexually-mature stage (Dietrich and Stöcker, 1968). Ontogenesis in the ectoparasitic mite *Varroa jacobsoni* takes place exclusively within sealed brood cells of its natural host *Apis cerana* as well as of the new one *A. mellifera.*

Beyond a pure scientific interest, knowledge of ontogenesis particularly of parasites of domestic animals and/or of cultivated plants contributes to a better understanding of their population dynamics and hence it can be helpful to predict the appropriate time for treatment against parasites. Studies on ontogenesis of honeybee parasites could also contribute to the development of breeding programmes for resistant bee colonies against them.

Stages of ontogenesis

By (i) artificially infesting fresh sealed honey bee brood cells *in situ* with adult female *Varroa*-mites, (ii) marking the position of the infested cells of the brood comb on a transparent sheet and (iii) putting the brood comb back to the bee colony again until the time of observations, it was possible for the first time to recover the concrete mites and to observe the development of their descendants under natural conditions and at different stages of development of the host (Ifantidis, 1981, 1983; Martin 1994).

The period between hatching from egg and reaching the adulthood in *Varroa* mite is subdivided in proto- and deuto-nymph stages (Fig.1). Each stage (I and II) consists of a mobile and an immobile (pharate) phase (Ifantidis, 1983; Laurent and Santas, 1987; Accorti and Nannelli, 1990; Donze and Guerin, 1994). Sakai *et al.* (1979) proposed the term *"chrysalis"* (CHR) for the pharate phases. This term is accepted by the author of this article and is used here more often for the *second* pharate phase, characterised as *"deutochrysalis"* (DCHR). Each CHR phase is terminated with the rejection of a skin (exuvium). The exuvium, especially of female DCHR, is very discernible (Fig. 2) and can be used to calculate the number of new adult daughters in multi-infested infested cells (Ifantidis, 1984; Martin, 1995a).

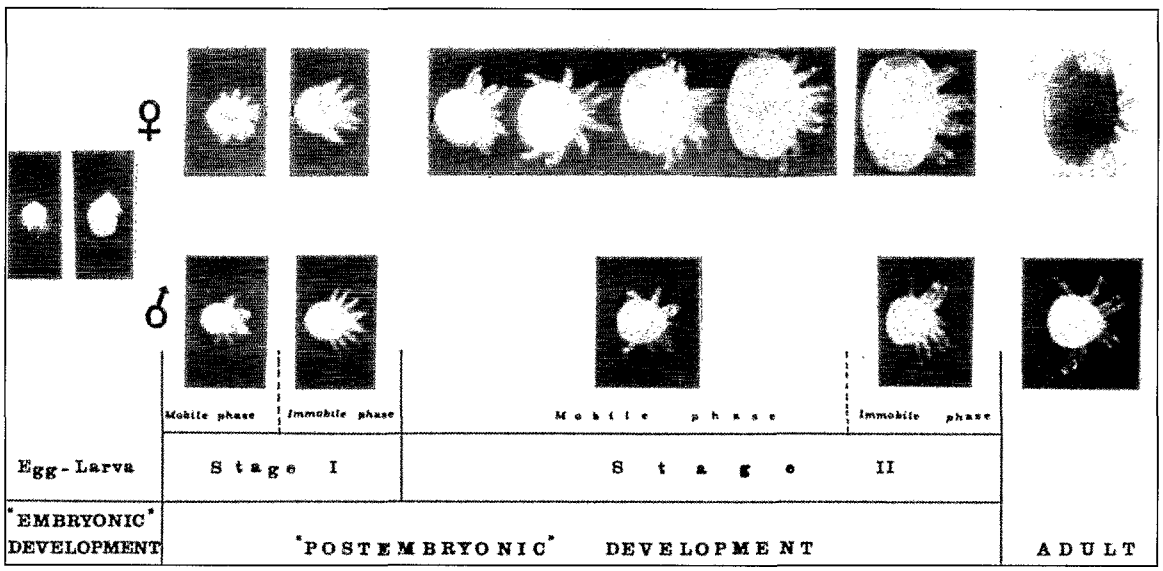

Fig. 1. Ontogenetic development of *Varroa* mite (Ifantidis, 1983).

Fig. 2. A fresh emerged female *Varroa* mite with the skin of DCHR. The meandering symmetrical white Malpigian tubules are well visible along the distal margin of the opisthosoma (Original).

Duration of ontogenesis

In *V. jacobsoni* the formation of the larva takes place within the egg shell and may be observed few hours after the depositing of the egg (Nannelli, 1985). Also the next developmental stages in this mite are accomplished within a relative short time. On average, the whole period from egg-larva to adult lasts 5.8 and 6.6 days for female and male mites respectively (Rehm and Ritter, 1989; Ifantidis, 1990; Donze and Guerin, 1994; Martin, 1994).

The duration both of female and male proto- and deuto-nymph of the mite is accurately given by Martin (1994). Donze and Guerin (1994) give more details by measuring separately the duration of mobile and pharate phases. In this connection it is interesting to note that the duration of *male DCHR* is 10 to 15 hours *shorter* than the corresponding phase for the female (Ifantidis, 1983; Donze and Guerin, 1994). On the contrary, male mobile protonymph (Donze and Guerin, 1994) has a period about two time *longer* than female. The final result is that the *whole* ontogenetic period is *longer* in the male than in the female (Donze and Guerin, 1994; Martin, 1994).

In any case, the very fast ontogenesis of *Varroa* mite *"is partly acquired by nutrimentary oogenesis"* (Alberti and Zeck-Kapp, 1986). Akimov and Yastrebtsov (1984) have found that the egg of this parasite expands rapidly during vitellogenesis. According to Steiner (1993) the (first) egg doubles in size between 20 and 35 hours post capping (hpc).

Morphology of developing mites

In the *Varroa* mite a clear *sexual dimorphism* is very obvious already from the second pharate phase of ontogenesis (Ifantidis, 1983).

Macroscopically the distinction between sexes is based firstly on body *size* as well as on relations among sizes of different parts of the body (see Fig. 1). Males are smaller than females in all developmental stages, although the differences are less apparent during stage I. In addition, the legs in relation to the body size are longer in male than in females. A typical trait of CHR, both of males and females, is that their legs are more or less out-stretched forward.

A second macroscopical difference between sexes concerns body *shape*. With the onset of the mobile phase of stage II, females change from the oblong shape of the stage I to an egg shape which gradually becomes transversely elliptical. The final shape is obtained with the onset of the phase of DCHR. The definite shape of male is rather triangular.

Finally, differentiation between sexes exists also in connection with body *colour* but only in the adult stage, females being brown and males light yellow. Coloration starts in the form of a rose thin ring on the periphery of the opisthosoma of the moulting female DCHR (personal observation).

Timing of ontogenesis

In the case of Mesostigmata, a maturing oocyte inhibits the development of all the rest (Vitzthum, 1931). Subsequently ontogenesis shows a typical periodicity as it is demonstrated also in *Varroa* mite. The development of each descendant within a family starts on an average 30 hours after initiation of the ontogenesis of the previous one. The first descendant appears as egg-larva about 60-70 hpc both in worker and drone brood cells (Ifantidis, 1983; Accorti and Nannelli, 1990; Donze and Guerin, 1994; Martin, 1994).

Feeding behaviour

Donze and Guerin (1994) observing the whole ontogenesis within transparent polistyrol cells were able, beyond others, to describe feeding behaviour of *Varroa* mite. Mother mites normally establish one but occasionally also a second feeding site on the 5th sternit of the bee pupa. According to the authors this is very critical for the survival of all developmental stages, because their mouth parts are not strong enough to pierce even the relative soft cuticle of the yet white bee pupa. If for any reason the developing mite could not have the possibility to reach feeding site(s) it would die of starvation. The feeding site is important also for adult males, the chelicerae of which are transformed to spermadactyls.

Composition of *Varroa*-family

In a normal *Varroa* family only the first descendant is a male (Rehm and Ritter, 1989; Ifantidis, 1990). The earlier misinterpretation regarding sex sequence in the family of this parasite, according to which the unique male ought to be born second in the sequence of the descendants (Ifantidis, 1983), was based on three concrete facts: (i) The macroscopical differentiation of sexes is very clear only in the last pharate phase; (ii) The phase of DCHR is indeed *faster* in the male; (iii) the unique male and the first female reach adulthood almost simultaneously, i.e. about 230 hpc, male being preceding about 10 hours (Ifantidis, 1983; Donze and Guerin 1994; Martin 1994). So it was thought that the mite with the faster development ought to be born at the second position. This is true but it is valid for females, not for males!

A mother mite can produce a maximum of seven offspring in drone cells and six in worker cells, with 5-6 and 4-5 being the norm respectively (Ifantidis, 1984; Martin, 1994). A normal family appears in the 77 to 78% of the observed cases of worker brood in the European races of *A. mellifera* (Ifantidis 1983; Martin 1994). In the drone brood this percentage is 64% (Martin,1995b). The non normal families include parental mites which produce either only males (arrenotoky) or lay less than three eggs or also mites which start very late with egg laying in the invaded cell.

All stages of development of the parasite can be observed at the *same* time within a normal *Varroa* family in a brood cell because of the periodicity of ontogenesis (Fig. 3). This happens only when the invaded cell contains already a worker bee pupa with dark eyes, not earlier (Ifantidis, 1981, 1983). That is why only brood cells containing host of this age have to be examined, if the reproductivity of the parasite must be determined (Ifantidis, 1983; Martin, 1994).

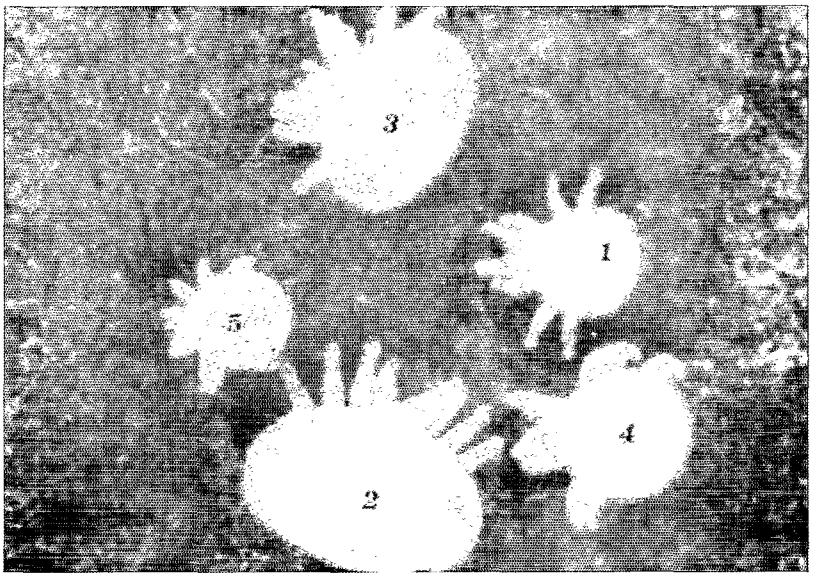

Fig. 3. Composition of a normal *Varroa* family in a sealed *worker* brood cell. The cell contains a pupa with dark eyes but yet white body. Numbering of mite descendants corresponds to the order with which they had been deposited as eggs. No. 1 is the unique male (from Ifantidis (1983) modified in relation to the sequence of sexes according to the findings of Rehm and Ritter (1989)).

Output of adult females

The number of mature daughters of *Varroa* mite produced in singly invaded cells is a very important parameter for the population dynamics of the parasite. Only adult females are considered to constitute the population of the parasite. Adult females are the long living individuals in this mite population. On the contrary adult males live for a few days. They usually die either in the yet sealed cell or shortly after emergence of the bee.

The number of mature female descendants per parental *Varroa* mite and per reproductive cycle (per entry of the mother in one brood cell) is obviously dependent at first on the duration of sealed stage of the brood of the host. This period is of 12 days as an average, especially for worker brood of European *Apis* races (Jay, 1963; Schousboe, 1990; Donze and Guerin, 1994). For African *Apis* races, for example *A. m. capensis*, this time is 11 days (Moritz and Jordan, 1992).

Within the period of sealed *worker* brood cell of European *Apis* races, the possibility exists also for the 4th mite descendant, i.e. for the 3rd female, to reach adulthood according to the parameters of ontogenesis mentioned already above. In addition, based on the percentage of normal families, the 3rd adult female ought to be produced in most cases of singly invaded worker brood cells. But the actual mean number of mature daughters per parental mite and per reproductive cycle is lower than the expected one: 1.3 (Schulz, 1984), 1.14 (Büchler, 1990), 0.86 (Ifantidis, 1990), 1.45 (Martin, 1994).

On the other hand all offspring of a normal egg-laying mite in drone cells (five female descendants per reproductive cycle) have ample time to develop fully (Martin, 1995b). In this case the period of sealed brood is about 14 days (Dade, 1977; Donze and Guerin, 1994; Martin, 1995b). Again, the actual number of adult daughters per normal reproducing mother is lower than the expected one, i.e. 4.0 (Martin, 1995b). When the entire population of parental mites, and not only of the normally reproducing ones, is taken in account the mean number of viable female offspring per mother mite drops deeper, i.e. to 1.7 (Ifantidis 1984) or 2.2 (Martin, 1995b).

Juvenile mortality

The great difference between expected and actual average number of adult daughters per reproductive cycle of a parental mite could be attributed to the death of young mites or in other words to *juvenile mortality* (JM) in the *Varroa* mite (Ifantidis, 1984, 1991, 1994; Martin, 1994). Accurate data from Martin (1994) concerning worker brood cells show *"a dramatic increase in the mortality of the 3rd and 4th* (i.e. of 2nd and 3rd female!) *offspring"*. JM in drone brood cells is considerably lower than in worker brood, even when the estimation is made only for mother mites, which lay no less than five to six eggs (Martin, 1995b). According to the same author JM in drone cells is mainly referred to the 5th female descendant.

Signs of dead developing mites

At first, different heavy body *deformations* indicate death of individuals in every stage. Death of *intact mobile* phases is recognized simply by the lack of motion of the descendants in *fresh* brood comb samples.
Again in fresh samples but now for *intact* immobile female and especially for DCHR, death can be recognized by the following signs:

(i) Lack of the spontaneous and more or less periodically *peristaltic movements of Malpigian tubules* (personal observations). Malpigian tubules (Akimov and Starovic, 1983; Ruijter and Kaas, 1983) are well formed and easily visible through the transparent integument of DCHR and/or of *hatching* DCHR as well as of a newly hatched female adult (see Fig. 2).

(ii) DCHR which die during the moulting process can be recognized additionally by the lack of *local movement* of legs. The legs are no more out-stretched; they are contracted under opisthosoma.

(iii) If the mite dies at the end of the moulting process the skin is only partially rejected. In this case the dead mite is in fact an adult individual.

Possible causes of JM

Even if no other reason might exist for the death of developing mites within the sealed cell, ontogenesis of the parasite could be violently interrupted by adult bees in the abandoned cells.

The majority of dead developing parasites is observed during the few days before emergence of the host (Ifantidis, 1991, 1994; Martin, 1994). The following factors could be considered responsible for JD in *Varroa* mites in the sealed cells:

(i) Possible infectious *diseases*. (Radtke *et al.*, 1994).

(ii) For developing male mites, the death may be attributed to the *bee's movements* during pupation in drone cells (Donze and Guerin, 1994).

(iii) We verify the statement of Martin (1994) that *some* dead developmental stages contain no body fluids. This could indicate the existence of *cannibalism* in *Varroa* mites. But under direct observation no case of cannibalism has been revealed (Donze and Guerin, 1994).

(iv) *Starvation* due to food competition, mainly between mite descendants at the feeding site, is considered an important death factor (Donze and Guerin, 1994; Martin, 1994, 1995a, 1995b). Even if it does not concern death of developing mites, it is very interesting to note here that in Fig. 3 in Martins (1994) study, an amazing great percentage of the dead descendants concerns adult daughters. As it was already noted above, about 50-65% of the dead mites corresponds to 3rd female descendants. The author gives no adequate explanation for this. In any case, starvation could not be a cause of death for adult daughters, which can normally feed themselves.

(v) Especially for the death of DCHR, starvation obviously could not be considered a possible factor; DCHR do not need food. But it would be interesting to know which part of "deutonymphs" in Martin's (1994) data concerns only DCHR. It is reasonable to suppose that DCHR die by the influence of *chemical* factors, i.e. some yet unknown substances which might originate from the maturing bee pupa (Ifantidis, 1984, 1991, 1994). The corresponding experimental verification of this assumption is still lacking.

The following question must also be answered, especially in connection to the previously discussed case (v) of JD: (i) to what extent is JD an accidental event?; (ii) how often does it occur in a population of bee colonies (bee yard)?; (iii) are there any differences in the frequency of its occurrence between bee colonies of the same or of different bee races?

The same questions could also concern death of mobile developmental stages. But here, starvation is added as a death factor and complicates the matter.

An eventual genetical basis of JD in *V. jacobsoni* could permit the development of a selection programme for resistant honey bee colonies of European races of *A. mellifera* against the parasite. One of the topics in the three year EUROBEE research programme sponsored by the Commission of EU since 1994, and in which our laboratory also participates, deals with JD of this destructive bee parasite.

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