Apidologie (1989) 20, 339-343

Original article

Sequence of the sexes in the offspring of *Varroa jacobsoni* and the resulting consequences for the calculation of the developmental period

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(received 29 September 1988, accepted 6 March 1989)

Summary — Previous investigations into the reproduction of *Varroa jacobsoni* mites have been based on the premise that the first-laid egg develops into a female and the second into a male. With cytological examination of eggs we can prove that the first egg with n=7 is male and the second egg with $2n$=14 is female. With this knowledge, the data from Ifantidis (1983) regarding the minimum duration of development can be recalculated : 6.9 days for males and 6.2 days for females. Each female mite within the worker brood of *A. m. carnica* can produce, apart from 1 male, up to three adult daughters per reproduction cycle.

***Varroa jacobsoni* — reproduction cycle — sex sequence — development duration**

Introduction

Each fertile female mite of *Varroa jacobsoni* Oud. usually produces only one male amongst a variable number of females within one reproduction cycle (Ifantidis, 1983). Males develop from an unfertilised egg with a set of n=7 chromosomes. Females, on the other hand, show a set of $2n$=14 chromosomes (Steiner *et al.*, 1982; De Ruijter and Pappas, 1983).

Until now, investigations into the reproduction of *V. jacobsoni* have always been based on the assumption that the first-laid egg was female and the second male. In these investigations either cytological determination of sex was not carried out (Ifantidis, 1983), or the methodology precluded establishment of the exact order of the egg being investigated (De Ruijter and Pappas, 1983).

In this work, the question of the sequence of the sexes within mite brood is clarified through chromosomal investigation of the first, second and third eggs. Further, the consequences for calculation of the developmental period of male and female offspring are discussed.

Materials and Methods

To carry out the experiment it was necessary to determine each stage of the bee brood in which the mite eggs were laid. Different times for the beginning of egg-laying are given in the literature (Ifantidis, 1983; Laurent and Santas, 1987). Therefore, using workerbrood of *Apis mellifera carnica* the investigations of Ifantidis were repeated and the data confirmed, *i.e.* the first egg is laid 60 h after cell capping and the further eggs follow at 30-h intervals.

In autumn 1988, three different groups of adult *Varroa* females were collected from sealed worker cells of *A. m. carnica* (Table I) :

Group A - mites about to lay their first egg,

Group B - mites about to lay their second egg,

Group C - mites about to lay their third egg.

The mites were removed from the brood cells just prior to oviposition and were dissected in Shen solution (Nicoletti, 1959). By pressing on the distal end of the genitoventral shield, the eggs could easily be removed through the oviporus.

The eggs were treated according to the following method described by Steiner *et al.* (1982) :

— Laid in freshly prepared 1% natrium-citrate solution with 0.005% colchicine for one hour and then transferred to 1% natrium-citrate solution for 10 min,

— Fixed in methanol/acetic acid (3:1) for 30 min,

— Dissociated on slides with one drop of 60% acetic acid,

— Spread with methanol/acetic acid (1:1),

— Air dried and stained with Giemsa.

According to Ionescu-Varo and Suciu (1979), embryonic development begins before the eggs are laid. Microscopic examinations of the preparations showed that all eggs were at a stage of multicellular embryos.

The results for the first, second and third eggs were each checked with a χ^2-test against a 50:50 distribution (Sachs, 1984).

Results

The results of the cytological examinations of the eggs dissected from the different mites indicate that the first egg is more frequently male and the second and third eggs are female (Table II).

Discussion

Assuming that the first egg is female and the second egg male, Ifantidis (1983) cal-

Table I. Description of the brood cells used.

Cell	*Group*		
	A	*B*	*C*
Stage of bee brood (Rembold *et al.*, 1980)	PP	PP	Pw
Adult mites	1	1	1
Offspring No.			
1	—	egg/larva	protonymph
2	—	—	egg/larva
Prepared embryo No.	1	2	3

Table II. Chromosome complements of the first, second and third eggs.

	Chromosome complement		
	n = 7	2n = 14	
Order of eggs	*No. of eggs*		*Significance*
1	17	1	$P < 0.01$
2	3	15	$P < 0.05$
3	0	8	$P < 0.05$

culated that *V. jacobsoni* females develop from egg to adult within 7.5 days and males within 5.5 days. This method limits itself to describing the minimum duration of development. The resulting reproduction pattern is shown in Figure 1A for worker brood of *A. m. carnica*. From this figure it is evident that each fertile *Varroa* mite can produce a maximum of two adult female offspring per reproduction cycle. However, the observations of Schulz (1984) contradict this. In 12% of fertile

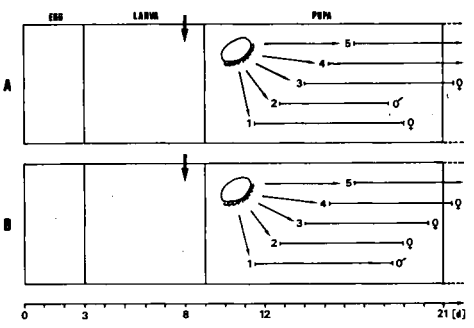

Fig. 1. Reproduction pattern for *Varroa jacobsoni* in the worker brood of *A. m. carnica*. A) Premise : the first egg is female B) Premise : the first egg is male.

mites in *A. m. carnica* brood cells of the Pd and Pdl stage he determined that three deutonymphs could develop into adults in the time remaining before emergence of the bee.

The order of the sexes is the fundamental basis for determining the duration of the development of the mite's offspring in the study of Ifantidis (1983). The present investigation shows that, as a rule, the first-laid egg is male and the second female, and therefore the developmental period should be re-calculated. On the basis of Ifantidis' data (1983), the minimum duration of the development from the egg to the adult may be determined as follows : 6.9 days for males and 6.2 days for females. The corrected reproduction pattern (Fig. 1B) shows that in the worker brood of *A. m. carnica* each female mite can produce up to three adult female offspring per reproduction cycle. The contradiction to Schulz (1984) can thus be dispelled. Whether or not the fourth egg can develop into an adult female is dependent not only on the duration of the postcapping period of the bee brood, but also variability in development time of the individual mite offspring.

Résumé — Ordre d'apparition des sexes dans la descendance de *Varroa jacobsoni* et les conséquences qui en découlent pour le calcul de la durée de développement. Dans les recherches effectuées jusqu'à présent sur la reproduction de *Varroa jacobsoni*, on considérait toujours que le 1er œuf pondu donnait une femelle et le 2e un mâle. Dans ces travaux, la détermination cytologique du sexe n'était pas faite (Ifantidis, 1983), ou bien la méthodologie excluait d'établir avec précision l'ordre des œufs étudiés (De Ruijter et Pappas, 1983). Afin d'éclaircir l'ordre d'apparition des sexes dans le couvain de l'acarien, on a étudié les chromosomes des 1ers, 2es et 3es œufs (Tableau I). On a pu ainsi démontrer que le 1er œuf était masculin avec $n = 7$ et les deuxième et troisième féminins avec $2n = 14$ (Tableau II). Puisque l'ordre d'apparition des sexes dans le travail d'Ifantidis (1983) était la base fondamentale pour déterminer les durées de développement, il est nécessaire de recalculer celles-ci en prenant en compte les résultats présents. Sur la base des données d'Ifantidis, la durée minimum de développement est de 6,9 jours pour les mâles et de 6,2 jours pour les femelles. Dans le couvain d'ouvrière d'*A. m. carnica* chaque femelle peut produire, outre un mâle, jusqu'à 3 descendantes femelles par cycle de reproduction (Fig. 1).

***Varroa jacobsoni* — cycle de reproduction — succession des sexes — durée de développement**

Zusammenfassung — Die Reihenfolge der Geschlechter in der Nachkommenschaft von *Varroa jacobsoni* und die daraus entstehenden Folgen für die Berechnung der Entwicklungszeiten. Bei den bisherigen Untersuchungen zur Fortpflanzung von *Varroa jacobsoni* wurde stets davon ausgegangen, daß sich aus dem zuerst abgelegten Ei ein Weibchen und aus dem zweiten eine Männchen entwickelt. Hierbei wurde entweder auf eine cytologische Bestimmung der Geschlechter verzichtet (Ifantidis, 1983), oder es war vom methodischen Ansatz her nicht möglich, die genaue Rangfolge der untersuchten Eier zu erfassen (De Ruijter und Pappas, 1983). Um die Reihenfolge der Geschlechter in der Milbenbrut zu klären, wurden in der vorliegenden Arbeit die Chromosomensätze definitiv erster, zweiter und dritter Nachkommen untersucht (Tabelle I). Es konnte nachgewiesen werden, daß das erste Ei mit n = 7 männlich, das zweite und dritte Ei mit 2n = 14 weiblich ist (Tabelle II). Da die Reihenfolge der Geschlechter in der Arbeit von Ifantidis (1983) eine wesentliche Grundlage für die Bestimmung der Entwicklungszeiten ist, wurden diese aus den Daten von Ifantidis unter Berücksichtigung der Kenntnisse dieser Arbeit neu berechnet. Es ergaben sich 6,9 Tage für die Männchen und 6,2 Tage für die Weibchen. In der Arbeiterinnenbrut von *A.m. carnica* kann somit pro Fortpflanzungszyklus jedes Weibchen neben einem Männchen bis zu drei adulte Töchter erzeugen (Abb. 1).

***Varroa jacobsoni* — Fortpflanzungszyklus — Reihenfolge der Geschlechter — Entwicklungszeit**

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