

Changed *Varroa jacobsoni* reproduction in *Apis mellifera* colonies in Java

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Summary — An update on the incidence and reproduction of *Varroa jacobsoni* Oud in sealed brood cells of *Apis cerana* F and *A mellifera* L colonies in Papua New Guinea (PNG) and Irian Jaya during 1994–1995 and in Java during 1992–1995 is reported. Reported also are the observed incidences and reproductive behaviours during 1995 of *V jacobsoni* in *A cerana* colonies on the Indonesian islands of Biak and Yapen located close to Irian Jaya and in *A mellifera* colonies introduced to those islands from Java during early 1995. In every *A cerana* colony examined regardless of location, female mites were only observed reproducing in drone cells. In the *A mellifera* colonies in PNG and Irian Jaya, there were no signs that female mites were reproducing in either worker or drone cells. This was also the case in *A mellifera* colonies reported from Java in a previous study in 1991 and in worker cells in Java during the present study in 1992. However, in *A mellifera* colonies in Java, each year subsequent to 1992, female mites were observed producing eggs and offspring in sealed brood cells. Reproducing female mites were also observed in both worker and drone cells in the *A mellifera* colonies that were introduced to Biak and Yapen from Java during January 1995.

Apis cerana / *Apis mellifera* / *Varroa jacobsoni* / reproduction

INTRODUCTION

Reproduction of the ectoparasitic bee mite *Varroa jacobsoni* Oud on the European honey bee *Apis mellifera* L may be affected by several factors including, mite infertility resulting from unknown causes (Ifantidis, 1983; Kulinčević et al, 1988; Fuchs, 1994), duration of the bee development period (Ifantidis, 1983, 1984; Rehm and Ritter 1989), lack of access by female mites to adult bees before invasion of brood cells

(Beetsma and Zonneveld, 1992) and the bee stage present when female mites enter brood cells to begin reproduction (Steiner et al, 1994). However, even though these and other factors may reduce a female mites' reproductive potential, none have been reported to result in total non-reproduction of all female mites in an individual bee colony or in groups of bee colonies from a particular locality.

Hence, the reproductive behaviour of *V jacobsoni* as reported by Anderson (1994) in

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Papua New Guinea (PNG) from 1991 to 1993 and in the Indonesian Provinces of Irian Jaya and Java during 1991 was startling in that, even though adult female mites were found to reproduce normally on drone brood of their natural host, *A cerana* F, and could readily spread from colonies of that bee to neighbouring *A mellifera* colonies, they totally lacked the ability to reproduce on sealed drone or worker brood following invasion of the *A mellifera* colonies. Since those studies, we have continued to monitor the mites' reproduction in *A mellifera* and *A cerana* colonies in PNG, Irian Jaya and Java and report on those results here.

MATERIALS AND METHODS

The incidence of female *V jacobsoni* was determined in sealed worker and drone brood cells that contained only prepupae or older in *A mellifera* and *A cerana* colonies in PNG and mainland Irian Jaya during October 1994 and April, August and December 1995, in *A mellifera* colonies at Sukabumi, Java during January 1992 and February 1993, and in *A mellifera* and *A cerana* colonies at Sukabumi, Jogjakarta and Pati in Java during July 1994 and at Sukabumi and Pekalongan during November 1995, using methods described by Anderson (1994). In brief, this involved removing the wax cappings from sealed worker and drone brood cells and visually examining them for female mites. The presence of *V jacobsoni* eggs, proto- or deutonymphs in cells or on the body of bee brood confirmed that the female mites were reproducing. In addition, we similarly determined the incidence and reproduction of female *V jacobsoni* in *A cerana* and *A mellifera* colonies on the Indonesian islands of Biak and Yapen located close to mainland Irian Jaya, after viewing evidence that *A mellifera* colonies had been introduced to those islands from Jogjakarta, Java during January 1995. The imported *A mellifera* colonies were accompanied by an official animal quarantine health certificate (no 6104/KH.330/SH.30/0195) issued by the Republic of Indonesia Department of Agriculture, Centre for Agriculture Quarantine, 5 January 1995. The examinations on Biak were conducted during April, August and December 1995, while those on Yapen were conducted during April and

August 1995. To help clarify aspects of *V jacobsoni* reproductive behaviour in *A mellifera* colonies in Java prior to 1992, we have also included in this paper the results reported by Anderson (1994) of *V jacobsoni* reproduction in four and five *A mellifera* colonies at Pekalongan (Central Java) and Sukabumi (West Java) respectively during October 1991. A total of 1 600 worker cells and 19 drone cells were inspected at Pekalongan of which 11 and 1 respectively were found to be infested with *V jacobsoni*, while 2 151 worker cells and 215 drone cells were inspected at Sukabumi of which 14 and 6 respectively were found to be infested with *V jacobsoni*. The mite infestations at each site were also not restricted to a single colony (see table II).

All the *A mellifera* colonies examined were housed in wooden 8 or 10-frame, single or double storey Langstroth hives. They had been present at the different sampling sites for at least 3 months prior to being examined for mites and, during this period, had not had honey extracted or brood removed. Furthermore, all colonies had no known previous exposure to chemical acaricides, the exception being those colonies examined in Java during 1995 which had been treated 8 weeks earlier with formic acid. The *A cerana* colonies examined in PNG, Irian Jaya, Biak and Yapen were all feral, while those examined in Java were hived in either wooden boxes like those described by Punchihewa (1994) or in hollowed-out coconut tree trunks. None had been previously exposed to chemical acaricides.

RESULTS

V jacobsoni infestations observed in *A cerana* and *A mellifera* sealed worker and drone brood cells at the various localities are summarised in tables I and II respectively. Also included in table II are details of *V jacobsoni* infestations observed by Anderson (1994) in sealed worker and drone brood cells in *A mellifera* colonies in Java during 1991.

In *A cerana* colonies at each locality proportionally more drone than worker cells were infested with adult female mites, but only drone cells contained reproducing female mites.

Table I. Numbers of sealed worker and drone cells infested with adult female *V jacobsoni* (FVJ) and reproducing female *V jacobsoni* (RFVJ) in *A cerana* colonies in Papua New Guinea (PNG), Irian Jaya, Java, Biak and Yapen.

<i>Location</i>	<i>Year</i>	<i>No of colonies examined</i>	<i>No of sealed cells examined worker:drone</i>	<i>No of cells with FVJ worker:drone</i>	<i>No of infested cells with RFVJ worker:drone</i>
PNG	1994	3	1 200:44	38:19	0:16
PNG	1995	8	2 750:103	82:62	0:57
Irian Jaya	1994	1	450:15	17:5	0:5
Irian Jaya	1995	21	6 140:374	392:153	0:141
Java	1994	10	4 000:322	54:62	0:56
Java	1995	4	1 280:51	24:17	0:16
Biak	1995	2	800:10	51:4	0:3
Yapen	1995	4	1 600:53	74:31	0:26

Table II. Numbers of sealed worker and drone cells infested with adult female *V jacobsoni* (FVJ) and reproducing female *V jacobsoni* (RFVJ) in *A mellifera* colonies in Papua New Guinea (PNG), Irian Jaya, Java, Biak and Yapen.

<i>Location</i>	<i>Year</i>	<i>No of colonies examined</i>	<i>No of sealed cells examined worker:drone</i>	<i>No of cells with FVJ worker:drone</i>	<i>No of infested cells with RFVJ worker:drone</i>
PNG	1994	14	7 000:619	68:33	0:0
PNG	1995	23	11 500:296	221:21	0:0
Irian Jaya	1994	4	2 000:127	33:5	0:0
Irian Jaya	1995	17	6 800:198	164:12	0:0
Java	1991*	9	3 751:234	25:7	0:0
Java	1992	5	2 500:0	10:0	0:0
Java	1993	5	2 500:0	193:0	135:0
Java	1994	4	400:23	14:15	11:15
Java	1995	6	2 100:95	235:27	181:23
Biak	1995	5	1 600:10	214:4	158:4
Yapen	1995	12	4 300:123	451:28	334:18

* These 1991 results are taken from Anderson (1994). See text for details.

In *A mellifera* colonies in PNG and Irian Jaya, proportionally more drone than worker cells were infested with adult female mites, but no signs of female mite reproduction were observed in either cell type. In *A mellifera* colonies in Java, even though the reproduction of adult *V jacobsoni* females in sealed brood cells during 1991 and 1992 was similar to that observed in PNG and Irian Jaya, female mites were nevertheless observed producing eggs and offspring in sealed worker cells during early 1993. Reproducing female mites were also observed in both worker and drone cells in *A mellifera* colonies in Java during 1994 and 1995 (tables I and II).

Finally, in *A mellifera* colonies that were introduced to the islands of Biak and Yapen from Java during early 1995, proportionally more drone than worker cells were infested with adult female *V jacobsoni*, with female mites producing eggs and offspring in both cell types.

DISCUSSION

Our observations of only non-reproducing adult female *V jacobsoni* in *A mellifera* colonies in Java during January 1992 and in New Guinea during 1994–1995 were similar to observations reported by Anderson (1994) from these same localities during October 1991 and throughout 1991 to 1993 respectively. However, our observations of increased incidences of adult female *V jacobsoni* in *A mellifera* colonies in Java during February 1993 and during each year thereafter, many of which were reproducing in sealed brood cells, clearly indicates that a change occurred in female *V jacobsoni* reproductive behaviour in *A mellifera* colonies in Java from that observed prior to 1993. The observed *V jacobsoni* non-reproduction in *A mellifera* colonies in Java prior to 1992 does not appear to have been caused from seasonal effects as the 1991

observations were carried out during October while the 1992 observation were conducted during January. In addition, the 1991 observations were reported from two widely distributed localities, Pekalongan in Central Java, and Sukabumi in West Java. This would seem to exclude the possibility that particular geographical phenomena caused the observed non-reproduction.

The cause of the observed change in *V jacobsoni* reproductive behaviour in *A mellifera* colonies in Java subsequent to 1992 remains unknown. However, we can offer two hypotheses. The first is that constraints that prevented female *V jacobsoni* in Java from reproducing in *A mellifera* colonies prior to 1992 may have ceased to exist some time between January 1992 and February 1993, thus allowing female mites to commence reproduction. The second is that a new isolate or 'strain' of *V jacobsoni* that was capable of reproducing in *A mellifera* sealed brood cells was introduced to Java between January 1992 and February 1993. It may be possible to experimentally verify this second possibility by testing for genetic differences between the female *V jacobsoni* we collected in Java prior to and subsequent of 1992. Perhaps such differences might be resolved by comparing specific DNA sequences from mites in the two populations and following methods described by Navajas et al (1994), as morphological and allozyme analyses have been found to be of limited value for detecting differences between *V jacobsoni* populations (Biasiolo, 1992; Delfinado-Baker and Houck, 1989).

Our findings of reproducing *V jacobsoni* in *A mellifera* colonies recently introduced to the islands of Biak and Yapen from Java indicate that environmental factors are unlikely to account for the observed non-reproduction of female *V jacobsoni* in *A mellifera* colonies in nearby Irian Jaya and PNG. These findings also increase the urgency for determining the cause of the *V jacob-*

soni non-reproduction seen in *A mellifera* colonies in Irian Jaya and PNG, as there is now a threat that the reproducing mite forms now present on Biak and Yapen may eventually spread to Irian Jaya and PNG, thus jeopardizing studies on the non-reproducing mite form. The spread of the reproducing mite forms to New Guinea would also bring them dangerously close to Australia.

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Résumé — Modification de la reproduction de *Varroa jacobsoni* dans des colonies d'*Apis mellifera* à Java. En complément à des études antérieures, la présence et la reproduction de l'acarien *V jacobsoni* dans des cellules de couvain operculé (pré-nymphes et stades ultérieurs) ont été étudiées sur des colonies d'*A cerana* et d'*Apis mellifera* en Papouasie Nouvelle-Guinée (PNG), à Irian Jaya en 1994 et 1995 et à Java de 1992 à 1995. Les mêmes données sont fournies pour les îles de Biak et Yapen, proches de Irian Jaya, i) pour des colonies d'*A cerana* indigènes et ii) pour des colonies d'*Apis mellifera* importées de Java début 1995. Dans toutes les localités il y avait proportionnellement plus de cellules de mâles que de cellules d'ouvrières infestées par des femelles adultes de varroa, que ce soit dans les colonies d'*A cerana* ou d'*Apis mellifera* (tableaux I et II). Dans toutes les colonies d'*A cerana*, indépendamment de la localité, les femelles de varroas ne se sont reproduites dans dans les cellules de mâles (tableau I). Pour les colonies d'*A mellifera*, en revanche, le résultat a varié selon la localité et l'année. En PNG et à Irian Jaya, les femelles de varroas ne se sont repro-

duites ni dans les cellules de mâles, ni dans celles d'ouvrières. À Java il en a été de même en 1992, ainsi que dans une étude antérieure faite en 1991. Mais en 1993, les femelles de varroas se sont reproduites dans les cellules d'ouvrières et les années suivantes, à la fois dans les cellules d'ouvrières et celles de mâles (tableau II). À Biak et Yapen également, en 1995, elles se sont reproduites dans les deux types de cellules. On propose deux hypothèses comme cause possible de ce changement. Selon la première, les contraintes qui empêchaient les femelles de *V jacobsoni* de se reproduire dans les colonies d'*A mellifera* à Java avant 1992 ont disparu entre janvier 1992 et février 1993, leur permettant ainsi de commencer à se reproduire. Selon l'autre hypothèse, une nouvelle souche de *V jacobsoni*, capable de se reproduire dans les cellules de couvain operculé, a été introduite à Java entre janvier 1992 et février 1993.

Apis cerana / *Apis mellifera* / *Varroa jacobsoni* / reproduction / Java

Zusammenfassung — Veränderung des Reproduktionsverhaltens von *Varroa jacobsoni* in *Apis mellifera*-Völkern in Java. In Fortsetzung früherer Untersuchungen wird über Vorkommen und Reproduktion der parasitischen Bienenmilbe *Varroa jacobsoni* in verdeckelten Brutzellen (Vorpuppen oder ältere Brutstadien) von *A cerana* F und *A mellifera* L in Papua Neu-Guinea (PNG) und in Irian Jaya während der Jahre 1994-95 und in Java während der Jahre 1992-1995 berichtet. Weiter wurden 1995 auf den nahe dem Festland von Irian Jaya gelegenen indonesischen Inseln Biak und Yapen vorkommende Völkern von *A cerana* sowie zu Beginn dieses Jahres aus Java dorthin eingeführte Völkern von *A mellifera* untersucht.

An allen Untersuchungsorten wurden sowohl bei *A cerana* als auch bei *A mellifera* die Drohnenbrutzellen stärker von adulten Milbenweibchen befallen als die Arbeiterinnenbrutzellen (Tabelle I, II). Unabhängig vom Ort reproduzierten die weiblichen Milben in den *A cerana*-Völkern ausschließlich in den Drohnenzellen (Tabelle I). In den *A mellifera*-Völkern in PNG und Irian Jaya gab es weder in Arbeiterinnenbrutzellen noch in Drohnenbrutzellen Anzeichen für eine Reproduktion der Varroamilben. Dieses Phänomen war in einer früheren Studie im Jahr 1991 auch in *A mellifera*-Völkern auf Java festgestellt worden, in der jetzigen Studie traf dies für Arbeiterinnenbrutzellen im Jahr 1992 ebenfalls zu. Im Gegensatz hierzu wurde in allen späteren Jahren ab 1993 beobachtet, daß die Milbenweibchen in den verdeckelten Zellen Eier und Nachkommen erzeugten. Reproduzierende Milben wurden ebenfalls sowohl in Arbeiterinnen- als auch in Drohnenbrutzellen in den im Januar 1995 aus Java auf die Inseln Biak und Yapen verbrachten *A mellifera*-Völkern beobachtet.

Für die Änderung des Reproduktionsverhaltens von *V jacobsoni* in den *A mellifera*-Völkern auf Java bestehen zwei Möglichkeiten. Nach der einen könnten zwischen Januar 1992 und Februar 1993 spezifische Bedingungen weggefallen sein, die die Vermehrung von *V jacobsoni* vor 1992 verhindern haben. Nach der anderen könnten zwischen Januar 1992 und Februar 1993 eine neue in Brutzellen von *A mellifera* reproduktionsfähige Abstammungslinie von *V jacobsoni* nach Java eingeführt worden sein.

***Apis cerana* / *Apis mellifera* / *Varroa jacobsoni* / Reproduktion / Java**

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