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Effect of mating frequency and brood cell infestation rate on the reproductive success of the honeybee parasite *Varroa jacobsoni*

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Abstract. 1. The reproductve biology of *Varroa jacobsoni*, whose females infest honeybee brood, was studied in natural and transparent artificial brood cells. These investigations were made under the headings of maturation behaviour and fertilization, and the influence of infestation rate of brood cells on the number of mated females produced per infesting *Varroa*.

2. Mating of *Varroa* daughters, observed in the transparent brood cells with time-lapse video, occurs just after ecdysis and as soon as they arrive on the faecal accumulation prepared by the mother. Such females are remated for as long as no other freshly moulted daughter arrives on the faecal accumulation.

3. The number of spermatozoa stocked in the spermatheca increases with remating, a strong indication for sperm mixing in this species when brood cells contain more than one *Varroa* foundress.

4. The number of daughters per infesting mother decreases at higher rates of infestation per cell, but the proportion of such daughters with a mate rises sharply due to the higher probability of finding a male within multi-infested cells. The number of mated daughters per mother is maximal in cells with two foundress *Varroa* females.

5. The frequency distributions of infesting mites in drone cells are aggregated, and approximate to negative binomial distributions.

6. We postulate from the above that the observed non-random infestation by *Varroa* in drone brood augments the mite's mean reproductive success through the production of a higher number of mated daughters than the corresponding Poisson distributions would.

Key words. *Varroa*, Acari, parasite, mating, reproductive success, infestation rate, *Apis*.

Introduction

Varroa jacobsoni Oud. (Acari: Mesostigma) is one of several mite species originally parasitizing the Asian honeybee *Apis cerana*. Since its transfer to the European bee, *Apis mellifera*, over 50 years ago, *Varroa* has caused severe damage to the new host populations, and has by now reached an almost worldwide distribution. *Varroa* females may reproduce several times in *Apis mellifera* capped brood (Schulz, 1984; Rosenkranz, 1993), and parasitize adult bees between reproduction cycles. *Varroa* maintains a strictly programmed clutch size and a precise sex ratio within the brood cell, independent of the number of foundresses. Oviposition starts at 60–70 h post-capping with a male egg (haploid), followed by four to five female diploid eggs laid at regular 30 h intervals (Ifanditis, 1983, 1991; Ifantidis & Rozenkranz, 1988; Rehm & Ritter, 1989; Martin, 1994; Donzé & Guerin, 1994). Thanks to this, the *Varroa* male matures first and the oldest daughter moults to adulthood some 20 h later. No reproduction occurs outside brood cells, because males do not survive on adult bees, so the duration of each reproductive cycles is limited by the duration of bee development, i.e. between capping of the cell and bee emergence. This lasts longer in drone than in worker cells so more offspring will consequently mature in drone brood. Asian bees remove almost all mites invading worker brood (Rath & Drescher, 1990; Tewarson *et al.*, 1992; Rosenkranz *et al.*, 1993), so reproduction is confined to the

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less cared for drone brood which is seasonal (Rath, 1992; Boecking & Ritter, 1994). The mite's population size oscillates between the beginning and end of the drone brood seasons (Fries *et al.*, 1994). On *A.mellifera* the mites invade both worker and drone brood and the *Varroa* population grows unchecked until the decline of the colony.

Protandry in *Varroa* enables the fertilization of a maximum number of daughters within the limited time span of the capped brood. For this, however, the newly emerged adult daughters must encounter a male. However, adult males are scarce, occurring in only 60% of single infested cells due to developmental mortality (Fuchs & Langenbach, 1989; Often, 1991). Our observations on *Varroa* reproduction show that the infesting mother forms a rendezvous site with her faeces on the cell wall on which all mobile individuals aggregate, and on which matings preferentially occur (Donzé & Guerin, 1994). The *Varroa* mother also prepares a feeding site on the bee pupa which is used by all offspring including the male. In cells infested by more than one *Varroa* foundress no aggressiveness between them was observed, and members of different families construct and cohabit the above-mentioned structures. The increased number of progeny in such cells does, however, leads to competition at the feeding site (Donzé, 1995).

The influences of the number of foundresses and of developmental mortality on sex allocation in parasitoids have recently been modelled, albeit separately (Nagelkerke, 1993; Nagelkerke & Hardy, 1994; Heimpel, 1994), but both parameters prevail in a population. Since *Varroa* must multiply on a strictly delimited resources within the brood cell, it is not surprising that the number of adult daughters per female decreases as foundress number increases (Fuchs & Langenbach, 1989; Moosbeckhofer *et al.*, 1988; Eguaras *et al.*, 1994). Despite this, multi-infestated cells regularly occur. The common use of the structures within the cells by co-foundresses would appear to indicate that some advantages accrue from multi-infestation. Thanks to observations detailed here we demonstrate that aggregated infestation by *Varroa* of natural brood cells contributes to the occurrence of males, leads to an increase in remating of females, and, as a consequence, *Varroa*'s mean reproductive success.

Material and Methods

Behavioural observations. Artificial polystyrol cells (internal dimensions 5.1 mm × 14 mm long for worker and 6.7 mm × 16 mm long for drones) containing naturally reared brood and infested by *Varroa* within *A.mellifera* colonies were used for observations. After capping, the cells were transferred to a laboratory incubator maintained at 34°C, 60% r.h.. Humidified air (2 l min^{-1}) entered the bottom of the incubator, two small fans placed at the ceiling ensured an air mix. Considering the importance of geotaxis in *Varroa* (Donzé & Guerin, 1994), the cells were kept in the natural position and turned only occasionally for observations. Matings in naturally infested artificial cells were observed round the clock from time-lapse video recordings.

Mating and fertilization. We set out to estimate the possible influence of the frequency with which rematings occur on the number of spermatozoa stored in the spermatheca. This character was then used to estimate potential fecundity, because the number of spermatozoa contained in the spermatheca corresponds with the maximum number of eggs laid by a *Varroa* female and with the number of oocytes in the ovary, i.e. up to thirty (Akimov *et al.*, 1988; Alberti & Hänel, 1986; de Ruijter, 1987).

Young virgin female mites were obtained as follows: we introduced a healthy young worker pupa into polystyrol tubes (internal dimension 5.1 mm × 14 mm long) and separated leg pair III to permit access to the fifth segment where mites prefer to feed (Donzé & Guerin, 1994). Then three to five pharate deutonymphs were carefully deposited on their venter on the cell wall behind the pupa's abdomen. The tube was closed at the head with a natural cap and at the other end with parafilm. 12 h later one of these moulted virgin females and two males were introduced into new tubes with pupae. To assure the sexual maturity of the males, only those from natural brood cells which already contained one adult daughter were used. By means of direct observations we determined the number of complete matings (i.e. those which lasted at least 6 min) which occurred with each of the introduced virgin females. These females were isolated on bee pupae after (A) one complete mating, (B) two complete matings, and (C) as females which had spent 48 h with males. The number of spermatozoa in females from natural brood cells (D) was compared with those in the experimental ones. Sperm counts were made on all *Varroa* females 3 days after exclusion of males. For this, the female was maintained on its back in a ringer solution by piercing the base of the peritreme with a fine needle (0.25 mm). With a second needle, the genito-ventral and metapodal shields were torn out and the genital organs (ovary, lyrate organ and spermatheca) were retrieved from the haemocoel, isolated from the digestive organs, and transferred onto a glass slide with a Pasteur pipette. Having separated the spermatheca from the other organs, a slight pressure was applied to the cover slip to expel the spermatozoa from the spermatheca for counting.

Reproduction in natural brood cells. One empty drone or worker comb was introduced into separate *A.mellifera* colonies strongly infested by *Varroa* and the queen was enclosed on the comb for 48 h. Once adult bees started to emerge from these combs they were frozen. The age of pupae was determined on opening of the brood cells by their colour (Jay, 1962). All *Varroa* instars infesting these cells were determined (Ifantidis, 1983; Nannelli, 1986; Rehm, 1988; Milani, 1992). Exuviae were intensively searched for, and the number of infesting females per cell, as against female progeny, was estimated by subtracting the number of adult exuviae from the total number of adult females in the cell. In order to unify the data, we calculated the average probability for each immature *Varroa* to reach adulthood before host imaginal ecdysis (Fuchs & Langenbach, 1989). The reproductive success was thus analysed for every infested cell according to its rate of infestation.

Reproductive success and fitness. The exact reproductive rate of each infesting female could be ascertained only from single infested cells, because multi-infested cells can contain fertile and sterile infesting females simultaneously. Therefore our data for multi-infested cells are represented by a mean value for all foundressess in each cell. As males do not survive outside the brood, we consider the reproductive success in terms of daughters which have the opportunity to mate. Furthermore, fitness was calculated by assuming that: (1) all females have the same

clutch size, (2) all foundresses lay only one male egg, (3) all foundresses in cells without adult males have zero fitness, (4) offspring of all females have equal mortality rates, (5) all adult daughters have the same chance of mating, (6) the fitness criterion is phenotypic without consideration for the genetic system, and (7) inbreeding plays no role. To measure fitness (Nagelkerke, 1993) we take into count the total number of daughters (d) and males (m) as well as the mean number of males (m_f) and daughters (d_f) per mother in each infested cell.

$$F = d_f + m_f\,(d/m)$$

F is calculated for each cell and is the mean fitness gained per mother when $m > 0$; if $m = 0$ then $F = 0$ for all foundresses. The fitness gained at the level of granddaughters was not calculated since it depends of the frequency distribution of cell infestation.

Frequency distributions of infestation rates. The frequency of infestation classes (i.e. brood cells infested by 0, 1, 2, ..., 7 and >7 *Varroa* foundresses) was recorded from brood sampled in the same beehouses as above but in a greater number of colonies. Distributions of mites were sampled in each of twenty-eight drone brood combs from twenty-eight beehives in May and in sixteen worker combs from sixteen colonies in July. The observed frequency distributions were first compared with the Poisson distributions (i.e. random distribution) for the same mean infestation rates (MIR), defined as the total number of infesting mites divided by the total number of cells opened (V/C), with the Chi-square test.

As many quantitative analyses on host–parasite relationships have shown that the frequency distributions of parasites can be described by the negative binomial distribution (Crofton, 1971; Anderson & May, 1985), we tested its goodness of fit for *Varroa*. The negative binomial distribution is defined by two parameters, i.e. the arithmetic mean of infestation (MIR) and the exponent k (Elliott, 1973). When k approaches 0 the degree of aggregation increases, and k approaches ∞ for a Poisson distribution. We calculated the expected values of the negative binomial distributions corresponding to all our observed frequency distributions and the goodness of fits were tested using χ^2.

Varroa reproductive success in drone versus worker brood. The observed infestation frequency of the cells was combined with the reproductive success of mites at different infestation levels (Table 1). The mean reproductive success per *Varroa* mothers per comb is provided by:

$$\sum_{i=0}^{\infty}(p_i \cdot RS_i \cdot i) \Big/ \sum_{i=0}^{\infty}(p_i \cdot i)$$

where p_i is the probability for brood cells containing i *Varroa* foundreses and RS_i is the average number of daughters per mother produced in cells with i foundresses.

Results

Mating and fertilization

Developing and mature offspring were observed preferentially on the faecal accumulation where 90% of mating events occur. We observed 287 mating events in 247 h of observation in six cells with mature daughters. The distribution of the mating duration is clearly bimodal with 70.5 ± 8.4% (mean ± SD) lasting less than 3 min and 25.8 ± 8.2% (mean ± SD) lasting longer than 6 min ($n = 6$ males). Since only the latter present the complete mating sequence we assume that sperm transfer only occurs in matings which last at least 6 min. Mature offspring undertake frequent rematings. After the arrival of a newly moulted daughter on the fecal accumulation ($n = 5$), the male no longer makes complete matings with the older females (Fig. 1A). When no additional daughter moults to adulthood, the male continues to mate with the sole one present ($n = 2$) (Fig. 1B). In cells with mature daughters, only 3.1% of matings occur with the mother and all were interrupted.

Successive newly-moulted mature females are afforded different durations of time for fertilization before bee emergence.

Table 1. Reproductive success of *Varroa* in worker and drone brood cells according to the rate of infestation, i.e. mothers per cell, percentage of infested cells containing mature daughters only without adult males, and those containing mature offspring of both sexes. The mean number of adult daughters per infesting *Varroa* is a global total for all infested cells, whereas the mated daughters are those present in cells where adult males were also observed. All data are the number of adult descendants expected at bee emergence. Values followed by the same letter are not significantly different (Student t-test for comparison between worker or drone cells at different infestation rates, $P < 0.005$, significance level corrected for multiple comparisons). Although data was not distributed normally at low levels of infestation, the t-test was still employed considering the high number of cells counted (central limit theorem).

	Worker brood				Drone brood							
	1*	2	3	4	1	2	3	4	5	6	7	>7
No. of infested cells	167	35	2	2	183	88	35	25	13	7	10	5
Per cent cells with adult daughters only	17.36	8.57	0.00	0.00	23.50	15.91	5.71	16.00	0.00	28.57	0.00	0.00
Per cent cells with males+females (= perfect cells)	46.11	80.00	100.00	100.00	36.61	75.00	94.29	84.00	100.00	71.43	90.00	100.00
Daughters/mother (mean)	1.07a	1.16a	1.46	0.84	1.62ac	1.67a	1.31ab	1.39ab	1.13ab	1.10bc	0.84b	0.99
'Mated' daugters/mother (mean)	0.83a	1.07a	1.46	0.84	1.11ab	1.46a	1.28ab	1.17ab	1.13ab	0.85ab	0.84b	0.99
Fitness gained per mother	1.66a	2.14a	2.92	1.68	2.20ab	2.93a	2.56ab	2.35ab	2.25ab	1.70ab	1.68b	2.00
Sons/mother (mean)	0.57a	0.54a	0.50	0.38	0.48a	0.53a	0.49a	0.45a	0.49a	0.48a	0.56a	0.22

* Infesting females per cell.

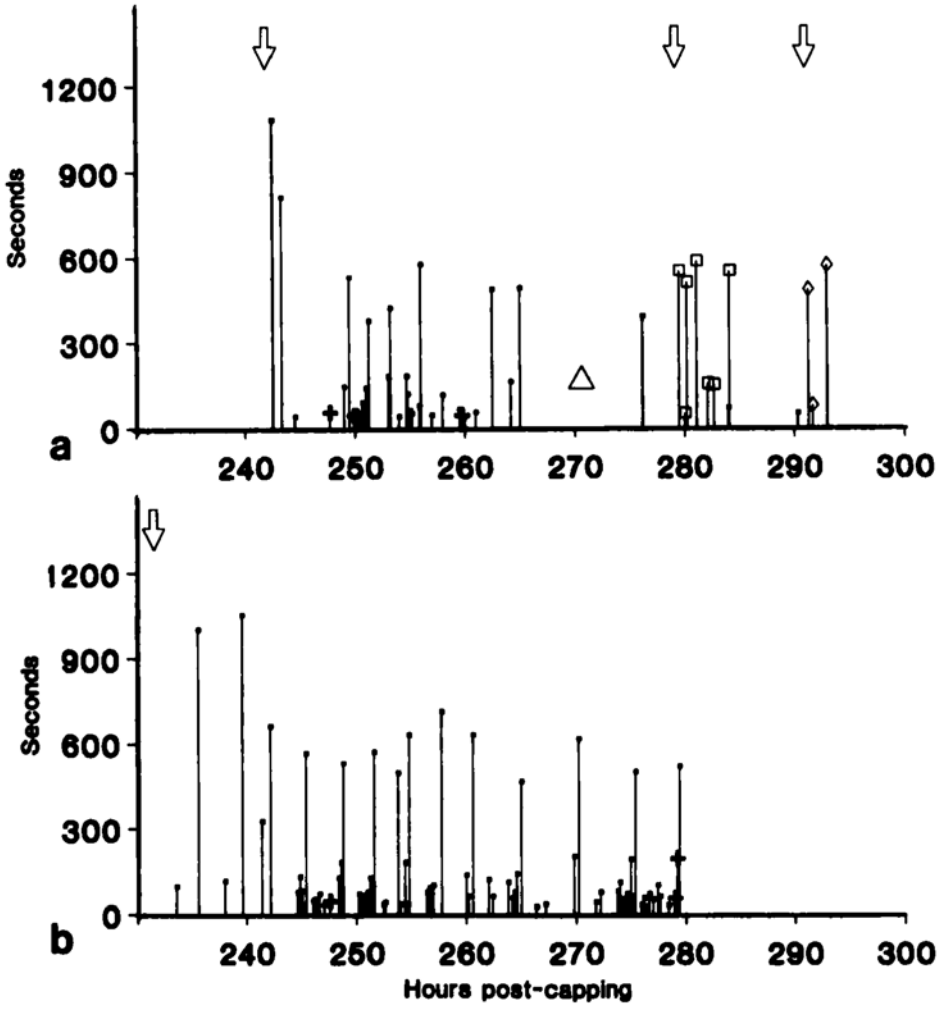

Fig. 1. Frequency and duration of matings between *Varroa* offspring observed in artificial worker cells. (a) Perfect cell where three daughters reached adulthood in succession. The single male reached adulthood at 222 h post-capping (h.p.c.). Bee imaginal ecdysis began at 294.5 h.p.c. Although frequent mating attempts occur which last less than 3 min, sperm transfer probably only occurs during sexual encounters lasting longer than 6 min (see text). The open arrows indicate imaginal ecdysis of the three successive daughters; ■ indicate matings with the first daughter, □ with the second, and ◊ with the third. ♀Represent mating attempts with the mother and Δ indicates an 8 h period without observation. (b) Perfect cell in which only a single daughter moulted to adulthood at 231 h.p.c. In this case fourteen complete matings occurred between the male and his sister but none with the mother. Observation was interrupted at 280 h.p.c.

In one brood cell with three daughters the first one was mated nine times within 37 h, the second four times in 12 h and the third twice in the 4 h period separating the mite's mobilization after moulting and the bee's imaginal ecdysis (Fig. 1). The round prospermatozoa have migrated to the spermatheca about 2 days after mating. One day later, they take on a fusiform shape and progressively the ribbon form (0.18–0.23 mm long). With increasing frequency of rematings the number of spermatozoa stored in a female's spermatheca increases (Fig. 2). No female which had been mated only once ($n = 5$) had spermatozoa, whereas nine of fourteen females mated twice stored between one and twenty-six spermatozoa. When mating proceeded *ad libitum* for 48 h the spermatheca contained over twenty-four spermatozoa in ten of eleven females dissected ($P < 0.0001$, df = 4, Kruskal-Wallis test). All pairwise comparisons between the different groups were statistically different ($P < 0.03$, $U > 12.5$, df = 1, Mann-Whitney U-test), except between females where matings proceeded *ad libitum* and those from natural brood cells ($P = 0.97$) (Fig. 2, groups C and D).

Reproduction in natural brood cells

Cells containing mature female offspring must be grouped in two categories (Table 1): those with one or several female daughters but without any adult male, and those named 'perfect cells' which contain mature offspring of both sexes. Cells without males account for, respectively, 17% and 23.5% of both single infested drone and worker cells. These proportions decrease at higher infestation rates. Perfect cells account for only 46% and 36.6% of the single infested worker and drone cells, respectively, but these proportions rise sharply with multi-infestation. In drone brood, the total number of daughters per mother which reach adulthood decreases with rising

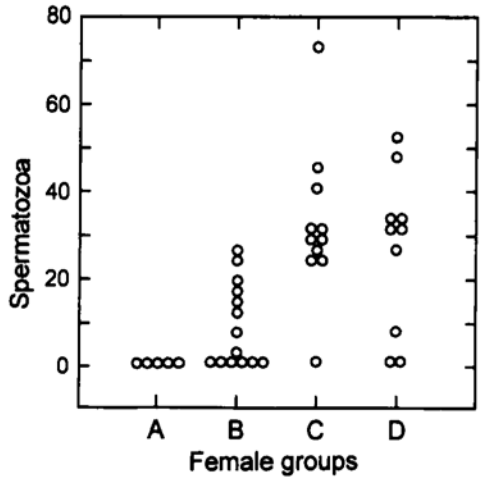

Fig. 2. Number of spermatozoa counted in the spermatheca of young *Varroa* females mated either once (A), twice (B), or *ad libitum* for 48 h (C) under experimental conditions (see text). Normally reproducing *Varroa* (D) were sampled in natural worker brood cells, some of which may have already reproduced. Each dot represents the number of spermatozoa counted in one female.

infestation rate. Counts were too low for worker brood to draw conclusions.

Not all young females have the oppurtunity to mate, because in cells without adult males no matings occur. Only daughters from perfect cells can be considered to determine the fitness or the effective reproductive success. But if the sex ratio in these perfect cells is too low, as for example in multi-infested ones, the male(s) present will not succeed in fertilizing the high number of daughters. Mating begins at some 230 h post-capping (h.p.c.) and ends at 280 h.p.c. in worker cells (330 h.p.c. in drone cells), and each male executes 0.3 ± 0.07 (mean ± SD, $n = 6$ males) complete matings per hour. In this 50–100 h period a male can thus undertake fifteen and thirty complete matings in worker and drone cells, respectively. If we assume that each female must be mated four times to acquire an adequate number of spermatozoa (Fig. 2), then a male could fertilize some 3.75 females in worker and 7.5 females in drone cells. From our data on 366 single and multi-infested worker and drone cells these values were exceeded only once in a double-infested drone cell where eight daughters were present for a single male.

Of the 1.62 daughters per mother in single-infested drone cells ($n = 183$) 1.11 daughters have the opportunity to be fertilized (here named 'mated' daughters) and 0.51 do not (Table 1). The fitness of the infesting females estimated at 2.2 corresponds to exactly double the number of the mated daughters (Table 1) because both values are only calculated for perfect cells with an average reproductive success per foundress. Since the proportion of perfect cells clearly increases with multi-infestation, a maximum number of mated daughters and fitness per infesting *Varroa* is reached in cells invaded by two mites. At higher infestation rates the number of mated daughters decreases due to the abrupt diminution in the total number of daughters per mother (Table 1). The global proportion of females' daughters which had no opportunity to mate due to the absence of a male was 15.1% ($n = 1091$) in the drone and 17% ($n = 276$) in workers cells. If we consider single-infested perfect cells only, we obtain 1.8 and 3.0 daughters per mother in worker ($n = 77$) and drone cells ($n = 67$), respectively.

Infestation pattern

From the foregoing, one could hypothesize that the average reproductive success of all infesting *Varroa* females will depend on their frequency distribution in brood cells. In worker combs, on the one hand, the mean infestation rate (MIR) varies from 0.09 to 0.82 *Varroa* per cell (V/C) and only eight of sixteen observed frequency distributions differ from the corresponding Poisson distributions for the same MIR (Fig. 3A). On the other hand, in drone cells the observed MIR is between 0.1 and 4.4 V/C, and twenty-four of twenty-eight observed distributions are aggregated and differ significantly from Poisson distributions. Compared to the Poisson distributions, these aggregated distributions are characterized by (i) a prevalence of infestation which is always lower, and (ii) a more frequent proportion of highly infested cells (Fig. 3b). The expected values of the best-fit negative binomial distributions do not differ from the observed values in twenty-three out of twenty-four distributions from drone brood, and in four out of six distributions in worker brood (for four drone and ten worker combs the χ^2 test was not applicable).

Aggregated distributions and reproductive success

We investigated the consequence of an aggregated distribution on the mite's mean reproductive success. In drone combs with MIR under 1 V/C there is little influence of the MIR on either the mean numbers of daughters/mother or on mated daughters/mother (mated daughter = 1.266 – 0.02 MIR, $n = 21$, $r^2 = 0.009$, $P < 0.7$). But when the MIR goes over 1 V/C, the reproductive success decreases (Fig. 4). Furthermore, when the reproductive success of the observed distributions and those of the equivalent Poisson distributions are compared, the plots show that the observed distributions lead to a higher number of mated daughters/mother when the MIR is lower than 1V/C (range 1.13 –1.38, mean ± SEM = 1.26 ± 0.01, $n = 21$) (Fig. 5). The mean reproductive success of the theoretical distributions increases regularly from 1.14 to 1.27 mated daughters per mother with a mean of 1.22 ± 0.04 (mean ± SEM, $n = 21$) when the MIR is lower than 1 V/C. It is worth nothing that the average reproductive success from the observed distributions of 1.26 approaches the maximum of 1.27 mated daughters per mother predicted by Poisson distributions (Fig. 5). However, when the MIR exceeds 1 V/C, the observed distributions lead to a lower estimated reproductive success than the Poisson distributions.

Discussion

Reproduction in natural brood cell

In this study a female infesting a cell alone has an average of 1.1 mature daughters in a worker cell and 1.6 daughters in a drone cell. These values are lower than those of either Fuchs & Langenbach (1989) or Otten (1991) who obtained 1.4 and 2.2

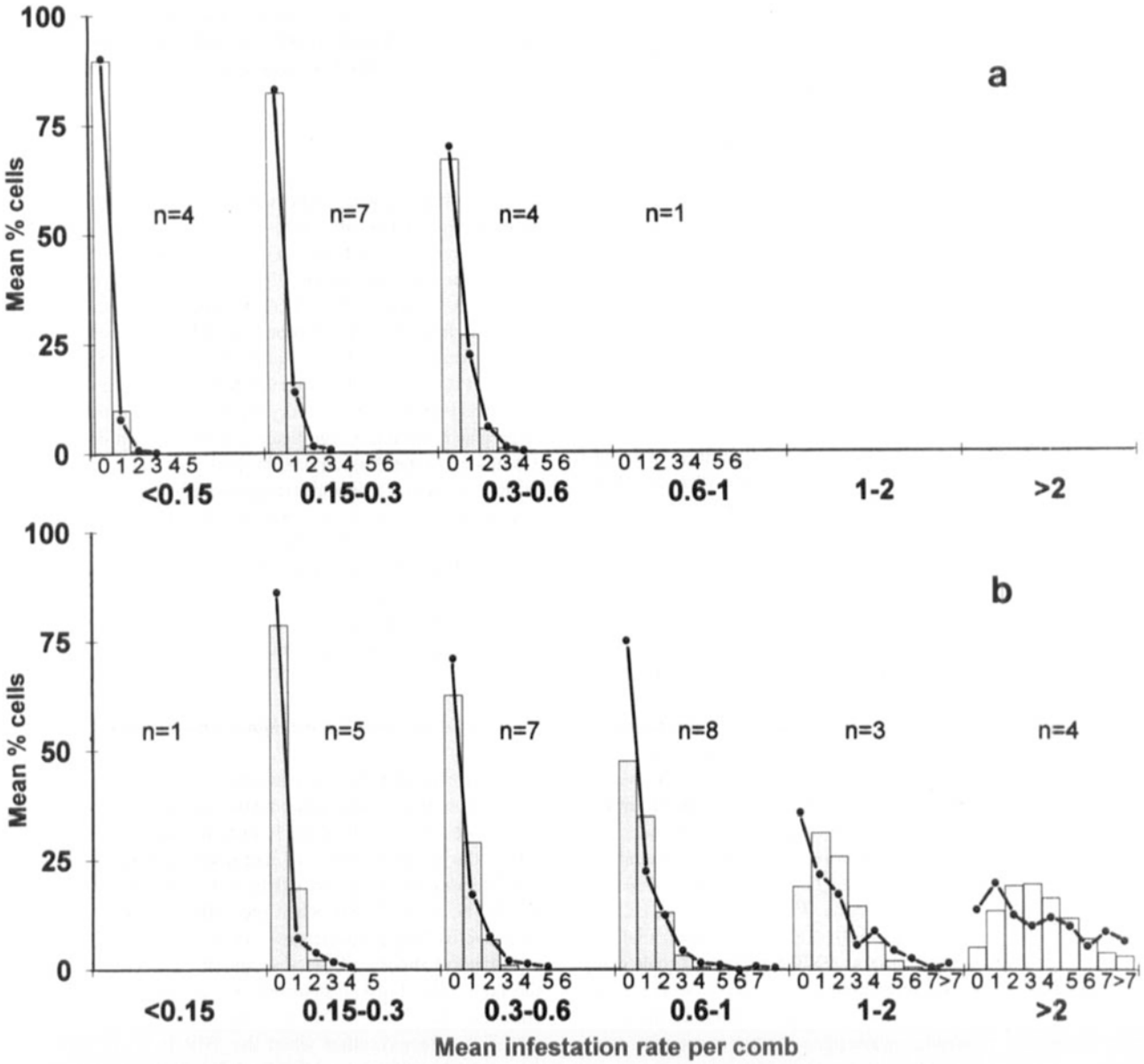

Fig. 3. Frequency distributions of cells infested by 0, 1, 2, 3, ..., 7 and >7 *Varroa* mites on worker (a) and drone combs (b) as related to the mean infestation rate (MIR) calculated for each comb by dividing the number of infesting *Varroa* mothers counted by the number of opened cells. Histograms represent mean expected values for Poisson distributions and dots (●) mean observed values for *n* combs.

daughters per mother, respectively, but higher for worker brood than those of Ifantidis (1984, 1991) who obtained 0.7–0.9 daughters. His value for drone brood (1.7 daughters per mother) is similar to ours. Although these variations could be due to bee genetic variation or climatic conditions, other factors could also influence the estimation at bee emergence. Firstly, in the cells containing older pupae it is possible to mistake mature daughters, which have darkened, for their mothers. We tried to avoid this by counting the exuviae. Secondly, not all descendants reach adulthood, such that the higher the age of the analysed brood the lower the expected reproduction rate will be, but the more accurate.

In all studies cited above the frequency of adult males is more constant and reaches about 0.6 males per infesting female. The rate of mortality at approximately 0.4 must be reduced by the proportion of non-reproducing mites, i.e. 0.15. Some causes for mortality of progeny can be deduced from observations on transparent artificial cells (Donzé & Guerin, 1994). In drone cells, the male protonymph becomes mobile during the prepupal stage but is rarely observed to feed on the prepupa. In contrast, later emerging female protonymphs have access to the prepared feeding site on the pupa and feed almost immediately. Apart from being starved for 25 h, the male protonymph can be squashed by the pupa's movements during pupation or be caught

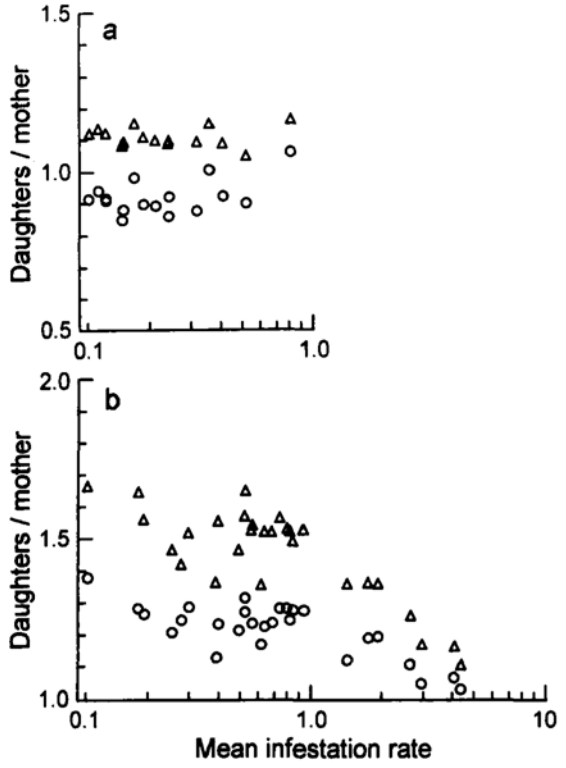

Fig. 4. Average reproductive success (daughters per mother) as a function of the mean infestation rate of brood cells by *Varroa* on worker (a) and drone combs (b). The reproductive success is calculated for each brood comb from the observed frequency distributions of *Varroa* mothers in brood cells, and the corresponding mean number of daughters and number of mated daughters per mother given in Table 1. Δ Is the mean number of mature daughters per mother per comb and ○ the mean number of mated daughters per mother. Each point represents data from one comb from different bee colonies.

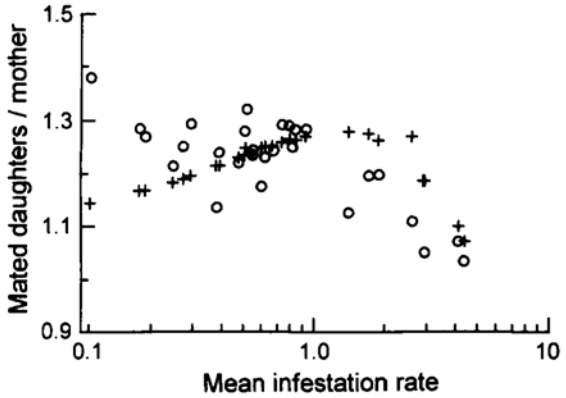

Fig. 5. Observed and predicted average reproductive success (mated daughters per mother) as a function of the mean infestation rate of drone brood cells by *Varroa*. The reproductive success is calculated for each brood comb from the observed frequency distributions of *Varroa* mothers in drone cells (○) and the corresponding Poisson distribution (+) combined with the mean number of mated daughters per mother given in Table 1. Each dot represents data from one comb from different bee colonies.

by the prepupal exuvium where a dead male nymph was discovered in several cells. In worker cells, by contrast, the male is still in the egg at bee pupation. Although this egg is carefully deposited in the protection of the cell apex, some are still disturbed by other mites in multi-infested cells or by the emerging appendages of the pupa. Moreover, the male protonymph always hatches in the anterior part of the cell after pupation and has to rejoin the posterior section to access the prepared feeding site (Donzé & Guerin, 1994). Some of these males do not find the only opening between the pupa's tarsi II and consequently die. Some *Varroa* males, which are haploid (de Ruijter & Pappas, 1983; Steiner *et al.*, 1982), probably also die in both male and worker cells because of a lethal genetic mutation (Smith & Shaw, 1980).

Although *Varroa*'s reproductive success is low compared to its potential reproduction, in the perfect cells 1.8 daughters per mother are produced in single infested worker cells and 3.0 in drone brood. These results are quite close to the potential reproductive success and higher than data recorded by other authors (Ifantidis, 1984; Fuchs & Langenbach, 1989). *Varroa*'s population growth is thus chiefly limited by the high number of sterile mites and by developmental mortality.

Varroa's mating and fertilization strategy

Since males do not survive outside capped brood, *Varroa* daughters must meet with males within the cell to be fertilized. Though the brood cell is a restricted environment, the available space is subdivided into two parts after pupation, a factor which could prevent encounter of the sexes (Donzé & Guerin, 1994). Arrestment by all stages on the faecal accumulation, outside of when they feed, assures the opportunity for rapid mating once mature. The high proportion of interrupted matings could reflect sperm depletion or another incapacity on the part of the male to complete the high number of attempted matings. We never observed aggression between males and have no evidence to suggest female guarding.

Mating encounters follow two patterns. First, as long as no other newly moulted daughter arrives on the faecal accumulation, matings occur with the resident female. Second, as soon as a new daughter arrives, mating proceeds almost exclusively with her (we observed only one complete mating with an older daughter in such a circumstance). In doing so, the males assure the transmission of the highest number of spermatozoa to a maximum number of newly moulted females in the limited time available. Several matings are needed to fill the spermatheca, which rarely contains over thirty-five spermatozoa.

We may ask what advantages rematings provide and whether in themselves they will not reduce the chances of fertilization of later moulting females. To explain frequent remating, two non-exclusive hypotheses are congruent with the biology of *Varroa*. Firstly, that rematings increase the level of fecundity, and, secondly, the occurrence of several males in multi-infested cells leads to competition between them. Our results indicate that rematings augment a female's fecundity. Since experimentally transferred females can lay up to thirty eggs during seven cycles (de Ruijter, 1987), the lifetime reproductive success of a female depends on the number of spermatozoa in her spermatheca. However, this success is also strongly dependent on two

factors. Firstly, the number of mated daughters may be limited in multi-infested cells where only a single male is present and where several females reach adulthood at the same time. Secondly, the real fecundity of females is strongly dependent on female survival in the beehive: the host defends itself and may kill mites (Rosenkranz *et al.*, 1993; Boecking & Ritter, 1994; Büchler, 1994). On *A.mellifera* 14% of marked *Varroa* females were observed to reproduce at least three times (Rosenkranz, 1993). Thus, in accepting a remating, both the male and female can hope to increase their lifetime fitness. Furthermore, we show here that *Varroa* can be polyandrous in multi-infested cells. The benefits of polyandry are controversial (Hardy, 1994). On the one hand, Ridley (1993) argued that polyandry could enhance the genotypic diversity of siblings which compete in one patch. On the other, polymating could decrease the effect of temporary sperm depletion in one male and therefore serve females in increasing the probability of obtaining sperm (Godfray, 1994). Females which accept rematings can expect to be provided with better sperm by an additional male and secondly hope to assure reproductive capacity of descendants by reducing their inbreeding. It is generally assumed that haplo-diploid populations do not suffer from inbreeding depression, for haploid subjects with a lethal mutation die. But, according to Helle (1965), inbreeding in arrhenotokous mites occurs and causes the viability of eggs to deteriorate. In the case of *Varroa*, genes may mingle within the population only within multi-infested cells. Therefore, to reduce inbreeding (since matings occur exclusively in the capped brood cells), *Varroa* permits the progressive stockage of spermatozoa from different matings and males in multi-infested cells.

Varroa's frequency distribution in cells and reproductive success

The infestation behaviour of *Varroa* on *A. mellifera* was studied by Fuchs (1985, 1992; Fuchs & Müller, 1988) who noted that at a low mean infestation rate (MIR) in worker brood the distribution of mites is approximately random, but not so in drone cells at a high MIR. From the same data, Reich *et al.* (1994) show that the infestation distribution of *Varroa* can be quite well fitted to a geometric distribution. However, these authors did not distinguish between the frequency distributions of infesting *Varroa* in worker and drone brood at similar MIR levels which he assumed to be equal. In this study we show that even at a low MIR the frequency distributions of mites are aggregated, but in drone cells only. Comparable aggregated distributions have been cited for a wide range of parasitic species (Anderson & May, 1978), such as ticks (Randolph, 1975) and helminths (Guyatt *et al.*, 1990; Maizels *et al.*, 1993). In helminths infesting humans the non-random infestation pattern is influenced by undefined genetic, immunological, ecological, behavioural or host social factors (Schad & Anderson, 1985). In the case of *Varroa*, infestation may be explained by the fact that the behaviour of the mite is influenced by volatiles (Le Conte *et al.*, 1989; Rickli *et al.*, 1992; Trouiller *et al.*, 1992) and contact chemostimuli (Rickli *et al.*, 1994) from larvae. Furthermore, the period for the cell invasion by *Varroa* on drone brood (40 h) is relatively long as compared to worker brood (20 h) (Ifantidis, 1988; Boot *et al.*, 1992). In addition, we show here that the infestation of drone larvae is not a random process, which may indicate individual differences between cells in the amount of stimuli triggering mite infestation. Furthermore, we cannot exclude that cells already invaded by mites become more attractive for some reason to other *Varroa*. Considering the small volume of the beehive, this may be the reason why such remarkably biased distributions arise in the relatively short periods of time available for cell invasion.

Although no precise data are available on the distribution of *Varroa* in *A.cerana* brood, we do know that the percentage of infested drone cells rarely exceeds 60% (Tewarson *et al.*, 1992; Anderson, 1994). From our data, a prevalence of 60% corresponds to 1 V/C, for when the MIR does not exceed this value the MIR and the percentage of infested drone cells are linearly related ($r^2 = 0.9$; $y = 0.05 + 0.016x$). If we assume that the infestation behaviour of *Varroa* is similar on *A.mellifera* as on *A.cerana*, then the data of these authors indicate that the MIR on *A.cerana* is below 1 V/C.

In *Varroa*, an advantage accruing from the biased distributions may be advanced on the basis that a maximum number of mated daughters is produced when two or three mites simultaneously reproduce in the same cell, but not all cells may be infested in this manner. As shown here, the strategy adopted by *Varroa* is more successful with an aggregated distribution than with a random one when the MIR stays below 1 V/C. At low MIR levels our data suggest rather predictable frequency distributions of the mites. The aggregation leads to an average reproductive success approaching the optimum predicted by the Poisson distributions. Thus, at the beginning of the brood season it is advantageous for the remaining *Varroa* in a hive to aggregate to increase reproductive success.

An ultimate explanation usually submitted for biased distributions of parasites is that they provide a partial refuge for the host, i.e. aggregated distributions ensure that an unexpectedly large number of hosts escape parasitization. This by-product of the aggregation ensures that the parasite has a host population on which to insure survival (Begon & Mortimer, 1986). Since *Varroa* exclusively infests the scarce drone cells of the original Asian host (Tewarson *et al.*, 1992; Anderson, 1994) and such infested drones produce fewer spermatozoa (Schneider & Drescher, 1988), the partial refuge would permit some drones to escape infestation and thus enable a higher quality of queen fertilization.

When mating is non-panmictic and progeny mate only at the natal site the mating structure involves local mate competition. When the number of foundresses is small the sex ratio is typically female-biased. Recent papers have explored the influence of developmental mortality on optimal sex ratio allocation and show that the risk of female virginity is correlated with the level of developmental mortality (Heimpel, 1994). Nagelkerke & Hardy (1994) stressed that only male mortality influences sex allocation strategy under local mate competition, and that the rate of mortality is more important for small clutch size. These models, however, are limited to a single foundress per offspring group. We show here that multi infestation can attenuate the negative effect of developmental mortality. However, clutch size must not lead to over-exploitation of limited resources. Therefore, to understand the evolution of a programmed sex ratio and small clutch size, the frequency distribution of foundresses in the brood must also be considered.

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