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Does time spent on adult bees affect reproductive success of Varroa mites?

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Abstract

Reproduction of Varroa jacobsoni Oudemans (Acari: Varroidae) and the number of Varroa mites that were found dead on the bottom board of the hive, were studied in relation to the period the mites spent on adult honey bees, Apis mellifera L. (Hymenoptera: Apidae), prior to invasion into brood cells. The maximum period on adult bees was 23 days. To introduce mites, combs with emerging worker brood, heavily infested with mites, were placed into a colony and removed the next day. At the beginning of the first day following emergence from brood cells, 18% of the mites introduced into the colony was found on the bottom of the hive. Part of these mites may already have died inside the capped brood cells, and then fallen down after cleaning of cells by the bees. At the second and third day following emergence, respectively 4% and 2% of the mites on adult bees at the previous day was recovered on the bottom, whereas from the fourth day on only 0.6% of the mites on adult bees was recovered on the bottom per day. After invasion into brood cells, 8–12% of the mites did not produce any offspring. Of the mites that did reproduce, the total number of offspring was 4.0–4.4 per mite during one reproductive cycle, part of which may reach maturity resulting in 1.2–1.3 viable daughters, and 8–10% of the mites produced only male offspring. Reproduction was independent of the period the mites had spent on adult bees prior to invasion into brood cells.

Introduction

Reproduction of Varroa jacobsoni, a parasitic mite on honey bees, only occurs on larvae and pupae in capped brood cells (De Jong et al., 1982; Ifantidis & Rosenkranz, 1988). Brood cells are invaded about 1–2 days preceding cell capping (Ifantidis, 1988; Boot et al., 1992). Approximately 60 h after cell capping the mites lay their first egg, which usually develops into a male. Subsequently, the mites continue to lay 4–5 female eggs in 30-h intervals. One or few of these female eggs reach maturity, the number depending on the development time of the bee pupa in the cell (Ifantidis, 1983; Ifantidis, 1984; Rehm & Ritter, 1989).

After emergence from brood cells, the female mites reside a certain period on adult bees in the colony before they invade a new brood cell (Boot et al., 1993; Boot et al., 1994b). The length of this period strongly affects the population dynamics of the mites, because mites cannot reproduce while they reside on adult bees and therefore reproduction is delayed. In addition, the period on adult bees may affect the population dynamics of the mites in two other ways.

Firstly, part of the mites will die during their stay on bees. Data on natural mortality of mites have been collected (Liebig et al., 1984; Rademacher, 1985; Imdorf & Kilchenmann, 1990), but in these studies the number of dead mites fallen down on the bottom of the hive has been studied in relation to the number of mites in the colony at the end of the experiments. During the experiments, the mites were distributed over adult bees and brood cells, and therefore data on relative mortality of mites residing on adult bees are still needed.

Secondly, the length of the period on adult bees may affect subsequent reproduction of the mites. Young Varroa mites that had been deprived of a period on
adult bees before introduction into a brood cell, laid fewer eggs and their oviposition was retarded (Beetsma & Zonneveld, 1992). In addition, Hänel & Koeniger (1986) found that after feeding on bees with relatively high juvenile hormone titres, the percentage of reproducing mites was higher than after feeding on bees with low titres. They hypothesize that Varroa mites take up juvenile hormone from the bees, which in turn stimulates invasion behaviour and subsequent reproduction of the mites. A longer period on adult bees may then well increase reproduction of the mites.

In this study we followed groups of Varroa mites starting at the moment of emergence from brood cells. Mortality and reproduction of the mites were determined in relation to the period the mites spent on adult bees.

**Materials and methods**

All experiments were done at a location isolated from other bee colonies by at least one km, in order to prevent exchange of mites between colonies (Sakofski & Koeniger, 1988; Rademacher et al., 1989; Greatti et al., 1992). Honeybees as commonly found in the Netherlands were used. These bees cannot be classified into a specific Apis mellifera race, because many races have been imported resulting in a mixed population. All brood was removed from the colonies and the mites present on bees were killed with 2–5 treatments of 15–25 ml 85% formic acid (Wachendorfer et al., 1985; Fries, 1989), until less than five mites were found on the bottom of the hive after treatment. Formic acid was chosen because its residue is probably the most transient when compared with other available acaricides. Since formic acid is a relatively weak method to kill Varroa mites, a rest population may have resided on the bees after treatment. Subsequently, a group of mites was introduced by placing heavily infested, emerging worker brood combs into the colony for one day. This group of mites was subsequently followed to determine mortality and reproduction in relation to the period spent on adult bees.

**Mortality.** Mortality was measured daily by counting of the mites on the bottom of the hive. At the end of the experiments the mites that had remained on the bees were killed by applying twice one ml of PerizinR in 50 ml water (Bayer; active ingredient: coumaphos), and the dead mites were counted. During the experiments, part of the mites invaded brood cells. Their number and the day at which they invaded were determined as well (Boot et al., 1993; Boot et al., 1994b). Therefore, the number of mites present on the bees at any day could be calculated by summing (1) the mites that fell on the bottom board from that day until the end of the experiment, (2) the mites that invaded brood cells from that day until the end of the experiment, and (3) the mites that still remained on the bees at the end of the experiment. For each day, the relative mortality of the mites was calculated by dividing the estimated number of mites on adult bees by the number of mites fallen onto the bottom board of the hive at that day. Subsequently, relative mortality of the mites was related to the period they had spent on adult bees.

The experiment to assess mortality per day as a percentage of the number of mites on adult bees, was repeated 17 times. On average, 769 mites (range: 280–1730) were introduced at the start of each experiment, and mortality was measured during an average of 13 days (range: 8–23). Mean mortality per day was calculated by weighing mortality in each experiment by the number of mites that were present on the bees.

**Reproduction.** A dated worker brood comb (Boot & Calis, 1991), containing worker larvae of 3–4 days old, was placed into the colony once a day to recapture the mites in brood cells. Worker brood cells are invaded from 15–20 h preceding cell capping (Boot et al., 1992). Therefore, the period that mites had spent on adult bees could be registered by marking capped cells daily on transparent sheets. After capping, the brood combs were moved into ‘nursing’ colonies for further development of the bee brood and the mites. Ten days after cell capping, when the honeybee brood was between 18 and 19 days old, the combs were taken out of the ‘nursing’ colonies and development of immature mites was stopped by putting the combs into the refrigerator. All cells were opened and the mites and their offspring were counted. Ten days after cell capping, young adult mites can still easily be distinguished from their mothers because their pigmentation is not completed yet (de Ruijter & Pappas, 1983). Subsequently, reproduction of the mites was related to the period that the mites had spent on adult bees.

The experiment to assess reproduction of the mites in relation to the time they spent on bees was repeated three times. In the first, second, and third series, offspring were counted of mites that resided up to 16, 20 and 10 days on adult bees, and reproduction was measured of 546, 663 and 473 mites respectively.
Table 1. Comparison of four reproduction characteristics between the three series. Data within a row with the same letter are not significantly different (Mann-Whitney U test for differences in number of offspring; Chi-square test for differences in fractions)

<table>
<thead>
<tr>
<th>Series</th>
<th>Series 2</th>
<th>Series 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting date</td>
<td>19-6-1989</td>
<td>31-7-1989</td>
</tr>
<tr>
<td>Length of period measured (days)</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>Total number of mites invaded</td>
<td>756</td>
<td>892</td>
</tr>
<tr>
<td>Number of cells where 1 mite invaded</td>
<td>546</td>
<td>663</td>
</tr>
<tr>
<td>Fraction of mites without offspring</td>
<td>0.12a</td>
<td>0.11a</td>
</tr>
<tr>
<td>Average number of offspring per reproducing mite</td>
<td>4.08a</td>
<td>4.00a</td>
</tr>
<tr>
<td>Average number of viable daughters per reproducing mite</td>
<td>1.23a</td>
<td>1.24a</td>
</tr>
<tr>
<td>Fraction of mites with only male offspring</td>
<td>0.08a</td>
<td>0.10a</td>
</tr>
</tbody>
</table>

Results

Mortality. The number of mites fallen on the bottom board of the hive was typically high during the first days after emergence from brood cells and then dropped to almost zero (Fig. 1). At the first day, during which the mites emerged from the brood cells were introduced into the colony, 17.8±5.4% of the number of mites that were estimated to be present on the bees fell on the bottom. Subsequently, 4.3±2.2% fell during the second day and 1.5±0.9% fell during the third day after emergence. From the fourth day onwards an average of 0.6±0.4% fell on the bottom board per day.

Reproduction. To compare reproduction of the mites only data from cells invaded by one mite were analysed because otherwise offspring of different mites cannot be distinguished, and reproduction of mites is negatively affected by mite density (Fuchs & Langenbach, 1989). Four characteristics of reproduction per brood cycle were analysed (Table 1): (1) the fraction of mites without offspring, (2) the total number of off-pring per reproducing mite, (3) the number of viable daughters per reproducing mite and (4) the fraction of mites with only male offspring. The number of viable daughters was estimated by pooling the young adults and immobile deutonymphs found per cell ten days after capping, since immobile deutonymphs can develop into adults within the remaining two days of honeybee development (Ifantidis, 1983). No differences between the series were found, except in series 3 for the higher number of offspring per reproducing mite when compared with series 1 and 2 (Table 1).

In none of the three series a significant correlation (Kendall Rank Correlation test) between time spent on adult bees and the four characteristics for reproduction was found (Table 2; Fig. 2), except for a negative correlation with the number of offspring per reproducing mite in the first series (Table 2). Detailed data are shown of the second series, because in this series offspring of mites was counted during a longer period than in the other series (Table 1).

Discussion

Mortality. Dead mites on the bottom of the hive are mainly found during the first days after emergence from brood cells. Part of these mites will already have died earlier inside cells, but they can only be recorded in the debris on the bottom from the moment the young bee has emerged and the cell is cleaned. Additionally, a considerable number of lightly pigmented adult mites were always found on the bottom during the first day after emergence. Such mites have probably just moulted into adults when the young bee emerges, and may be too young to survive on adult bees. After the first few days the number of mites that was daily found on the bottom board dropped to low levels. The few mites found may have died due to grooming activities by the bees (Ruttner & Hänel, 1992).

Mortality of mites on adult bees seems to have little effect on population growth, because only a few mites die per day, and because in colonies rearing brood the mean residence time of mites on adult bees is maximal-ly 1–3 weeks, depending on the number of brood cells available for mite invasion (Boot et al., 1993; Boot et al., 1994b). This may be different during winter in temperate climates, because long broodless periods may occur and mites may therefore have to stay on adult bees for several months. When the same percentage of mites dies per day during winter, even this low mortality may result in a high total mortality. Korpela
Fig. 1. Daily mortality of *Varroa* mites (% of the number of mites that was estimated to be present on the bees at that day) in relation to the length of the period spent on adult bees after emergence from brood cells. Values are based on mite counts on the bottom of the hive in 17 colonies.

Table 2. Correlation of the time that mites spent on adult bees prior to invasion into a brood cell with (1) the fraction of mites without offspring, (2) the average number of offspring per reproducing mite, (3) the average number of viable daughters per reproducing mite and (4) the fraction of mites with only male offspring (Kendall Rank Correlation Test)

<table>
<thead>
<tr>
<th></th>
<th>Correlation coefficient; significance level between brackets</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>series 1 (n=16)</td>
</tr>
<tr>
<td>Fraction of mites without offspring</td>
<td>-0.31 (0.06)</td>
</tr>
<tr>
<td>Average number of offspring per reproducing mite</td>
<td>-0.54 (0.003)</td>
</tr>
<tr>
<td>Average number of viable daughters per reproducing mite</td>
<td>-0.33 (0.08)</td>
</tr>
<tr>
<td>Fraction of mites with only male offspring</td>
<td>-0.13 (0.42)</td>
</tr>
</tbody>
</table>
et al. (1992) estimated a total mortality of 40% over a broodless period of 125 days during winter in Finland, which corresponds to a mean mortality of 0.4% per day. This is in the same order of magnitude as the 0.6% per day found in this study. Therefore, there is no reason to think that mortality of mites on adult bees depends much on the time of the year.

When mortality of mites is determined, it is generally assumed that the number of mites fallen down on the bottom of the hive provides a good estimate of total mortality, mainly because it was never measured how many mites disappear from the colony. Mites may disappear when they reside on bees that die outside the hive and this may have influenced our results. The number of mites lost in this way may be small, because the mites prefer young bees (Kraus et al., 1986), which remain mostly inside the hive, and because only part of the bees that do not return to the colony carries a mite. It is unlikely that mites disappeared by removal of debris including dead mites, as a result of hygienic activities by the bees, because the debris fell through a gauze screen placed over the bottom of the hive. Therefore the bees could not reach the debris on the bottom.

**Reproduction.** Within a period of up to three weeks after emergence from brood cells, reproduction of Varroa mites proved to be independent of the time the mites stayed on adult bees (Fig. 2; Table 2). This is in agreement with Wendel & Rosenkranz (1990), who also found no effect. Consequently, the period mites need to stay on adult bees to stimulate reproduction (Beetsma & Zonneveld, 1992) is either relatively short, or this period is variable and only mites that are sufficiently stimulated invade brood cells. In the latter case it is implicitly assumed that invasion into brood cells is limited because part of the mites is not motivated to invade. However, recently we found that all mites present on adult bees are apparently motivated to invade within a day after emergence from a brood cell, and that invasion is limited by the number of brood cells available in a colony with a constant number of bees (Boot et al., 1993; Boot et al., 1994b). Because mites are apparently motivated to invade within a day after emergence, and because reproduction of the mites was not affected by the time spent on adult bees, a short period on bees seems to be enough to stimulate reproduction. In addition, de Ruijter (1987) showed that contact with bees is by no means indispensable for reproduction of the mites. In his experiments, the same mites were repeatedly introduced into freshly capped brood cells (up to seven times). Although the mites had no contact with adult bees between successive introductions, the number of eggs produced per mite remained the same.

Our results cannot refute nor confirm the hypothesis of Hänel & Koeniger (1986). They proposed a 2-step-model in which (1) mites are stimulated to invade cells by ingesting juvenile hormone from adult bees with a high titre and (2) oviposition of the mites is initiated when they ingest juvenile hormone from the larvae. However, assuming juvenile hormone affects invasion and reproduction, the relative short residence on bees can only be explained when mites quickly reach bees with high enough titres. Varroa mites indeed change quickly from one-day-old bees to older bees (Kraus et al., 1986; Le Conte & Arnold, 1987), which usually have a higher juvenile hormone titre (Fluri et al., 1982; Robinson et al., 1987).

The fraction of mites without offspring was in the same range as found in other studies from Western Europe. Schulz (1984), Moosbeckhofer et al. (1988)
and Fuchs & Langenbach (1989) found 16, 7 and 7% respectively, while in this study 8–12% was found. The 8–10% mites with only male offspring was rather high. In the above mentioned studies 6, 3 and 3% was found, respectively. Mites that produced only male offspring probably had not mated, since haploid eggs of Varroa mites develop into males (de Ruijter & Pappas, 1983). Our estimates of viable daughters, 1.23–1.25 per reproducing mite are low compared to the above mentioned studies: 1.6, 1.5 and 1.5 viable daughter per reproducing mite, respectively. However, both estimates of this study and of the other studies are extrapolations, because cells have to be opened before development has been completed. The developmental stage of the brood that was opened to determine reproduction of mites differs between studies, and therefore the extrapolated values of viable daughters per reproducing mite are hard to compare.

Otten (1990) found seasonal variability in reproductive success of Varroa mites. In his experiments in Germany, reproductive success increased from January to June/July and decreased from August to November. Our data, which were collected from 19 June to 15 September, show that reproductive success is constant in this part of the year (Table 1).

**Effects of time spent on adult bees on population growth of the mites.** The relation between the period that Varroa mites have spent on adult bees and mortality and reproduction, proved to be rather straightforward. In general, large numbers of dead mites are found on the bottom board only within the first three days after emergence from brood cells. In this period 22.2±5.6% of the mites that were estimated to have been present in the brood cells before, was found on the bottom of the hive in our experiments. This may be a tool for beekeepers to estimate infestation levels in their hives, because the number of mites on the bottom of the hive will be strongly associated with the number of freshly emerged mites. Thus, mortality is mainly related to emergence and not to the period mites spent on adult bees. In addition, this period does not affect reproduction of the mite. Although reproduction is not enhanced, mites may still reside for several weeks or longer on adult bees, which raises the question why Varroa mites do not invade earlier. In selecting brood cells, the mites do not walk across the comb, but have to be carried close to a cell before invasion occurs (Boot et al., in press). This limits the rate of invasion (Boot et al., 1994), which in turn limits population growth of Varroa mites, as they cannot reproduce while on adult bees.

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