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Behavioral attributes and parental care of *Varroa* mites parasitizing honeybee brood

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Abstract. *Varroa jacobsoni*, an ectoparasite of the Asian honeybee *Apis cerana*, has been introduced world-wide, and is currently decimating colonies of the European honeybee *Apis mellifera*. *Varroa*'s reproductive cycle is tuned to that of drone cells, those mainly parasitized in the original host. We describe here how a single fertilized female, infesting a brood cell, can produce two to four adult fertilized females within the limited time span of bee development (270 h in worker and 330 h in drone cells), despite the disturbance caused by cocoon spinning and subsequent morphological changes of the bee. From observations on transparent artificial cells we were able to show how the mite combats these problems with specialized behaviors that avoid destruction by the developing bee, prepares a feeding site for the nymphs on the bee pupa, and constructs a fecal accumulation on the cell wall which serves as a rendezvous site for matings between its offspring. The proximity of the fecal accumulation to the feeding site facilitates feeding by the maturing progeny. However, communal use of the feeding site leads to competition between individuals, and protonymphs are most disadvantaged. This competition is somewhat compensated by the timing of oviposition by the mites. Use of a common rendezvous and feeding site by two or more *Varroa* mothers in multiinfested cells may have developed from the parental care afforded to them as nymphs.

Key words: Acari – *Varroa jacobsoni* – Reproduction – Parental care – Behavior

Introduction

Varroa jacobsoni (Acari: Mesostigmata) was originally an ectoparasite of the Asian eusocial bee, *Apis cerana*, but is causing severe damage to honeybee colonies since its transfer to the European honeybee, *Apis mellifera*. *Varroa*'s entire life-cycle occurs in the beehive, and all stages are obligate ectoparasites feeding on haemolymph. Female mites reproduce within the concealed habitat provided by brood cells and parasitize adult bees between reproductive cycles. Such females quickly invade open brood cells, attracted by volatiles released by bee larvae prior to capping, and prefer the drone brood cells (Schulz 1984; Le Conte et al. 1989; Fuchs 1990; Rickli et al. 1992). When inside, the mite soon conceals itself in the jelly behind the larva (Ifantidis 1988; Boot et al. 1992). Normal reproduction in this species consists of producing one male and two to four females, fertilized by their brother, during the period between capping of the cell and emergence of the adult bee (Ifantidis 1983, 1990; Ifantidis and Rosenkranz 1988; Rehm and Ritter 1989). Griffiths (1988) and Akimov et al. (1988) reviewed the morphological adaptations of *Varroa* for its parasitic life in the brood cells. In *Apis cerana* the mite reproduces only on the less cared-for male brood, as most infested worker cells are detected by worker bees and eliminated from the hive (Tewarson et al. 1992); Asian bees engage in mutual grooming and even remove mites from colonies (Peng et al. 1987a,b; Rath and Drescher 1990). European bees do not undertake systematic cleaning (Peng et al. 1987a; Boecking and Drescher 1991, 1992), so once established in a colony the parasite population explodes by using all the available brood.

Other parasitic mite species also live in family groups and utilize well-defined habitats such as a mammal's nest (Radovsky 1985), woven cells on a leaf surface (Saitò 1983, 1986a), or cavities of the noctuid tympanic organ (Treat 1958, 1975). These habitats are structured in different ways by the mites, occasioning use of different sites for different behaviors. In some Tetranychidae, the mites' web or leaf surface is often used for distinct behavioral activities (e.g. oviposition, defecation, feeding). In some species, all instars accumulate their feces at a common site, presumably to prevent fouling of resources or to repel predators (Saitò 1983). The structuring of space and behavior reaches a high level in *Dichrocheles phalaenodectes* (Mesostigmata) as feeding, egg-laying and

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molting always occur in three different cavities of the noctuid tympanic organ. Feces, for example, are accumulated in the countertympanic cavity, and seem to have a biological significance as it is supposed that it is here that nymphs moult and adults mate. However, no direct observations were possible (Treat 1958, 1975). The interactions between cohabitants are limited to feeding at a common wound in the case of mammalian ectoparasites, which in fact permits nymphs to feed since they cannot pierce the host's skin when experimentally isolated (see Radovsky 1985). Saitò (1986a,b) shows that adults of the spider mite *Schizotetranychus celarius* manifest biparental care in the form of defence of the nest, and consequently the offspring, against predators.

At first sight the brood cell would appear to provide an ideal environment for reproduction in *Varroa*, with its stable temperature and humidity, accessible food source, and absence of predators or competitor species. However, the time available to *Varroa* is limited and the many changes occasioned by the bee's development limit the space available for the female mite and its family. From video observations on transparent polystyrol cells containing naturally infested brood from the beehive we set out to establish how the female succeeds in parasitizing the developing bee, structures the living space for its developing family, chooses sites for oviposition, and, in multiinfested cells, cohabits in these structures with members of other families. We wished to establish if nymphs could survive on the pupa on their own. Furthermore, using these observations we have endeavoured to elucidate the genesis and biological role of space and behavior structuring in *Varroa*.

Methods

Infestation of artificial brood cells. Artificial brood cells were infested naturally within *A. mellifera* colonies in cylindrical transparent polystyrol cells (internal dimensions: 5.1 mm diameter × 14 mm long for worker and 6.7 mm diameter × 16 mm long for drones) incorporated at an inclination of 5–10° in groups of 60–70 in normal wax combs and coated with honey. The dimensions of these polystyrol cells are similar to those of natural ones in our beehives (5.2–5.3 mm × 12 mm long for workers and 6.5–6.8 mm × 14–15 mm long for drones) and to those cited in the literature (5.1–5.4 mm for workers; Erickson 1990). The queen was confined to the artificial cells for 12 h in a beehive heavily infested with *Varroa*. Some 8.5 days later, the time of sealing of each cell was recorded at intervals of 1–2 h in an observation beehive. After capping, these naturally infested (0–10%) cells were removed for observation in an incubator at 34°C and 60% RH.

Direct observations of these cells were made with an operation microscope (Zeiss OPMI 1-FC) mounted with a black and white CCD video-camera (Canon Ci-20PR) connected to a time-lapse VHS recorder (Bischke UB-480). A cold-light source (300–500 lux) illuminated the cell, which was fixed by wax to a metal stem gripped in a lathe-holder. This allowed rotation in the longitudinal axis. To permit slight inclination of the cell, the holder was mounted on a ball-joint and this on a microscope plate which allowed displacement of the cell in the two horizontal axes. Simultaneous observation of the sides and back of the cell was made possible with two inclinable mirrors placed under the cell, such that three views of the cell were recorded, i.e., from above and from each side. All manipulations of cells and optical settings were done through two air-tight hand ports in the door of the incubator. The eyepieces of the microscope also passed through this door.

Observations on brood cells. Cells in which reproduction was observed to proceed normally (Fig. 1) were placed in their natural position (i.e., pupa on its back) under the microscope and observed round the clock by time-lapse video (24 or 3 frames sec^{-1}). Duration of development, feeding activity and utilisation of the available space in the cell by each individual was recorded. Interactions between individuals such as mating and competition for feeding were studied. Additionally, infested artificial cells kept in the incubator were observed one or more times daily. To confirm that results relating to position of first eggs and the feces were not artefacts arising only in the artificial cells, we observed the position of feces and the location of eggs and pharate nymphs in natural cells. Pearson's χ^2 and the Fisher exact test were used to compare frequencies. Throughout this article means ± standard deviations (SD) are indicated.

Track analysis. Records of *Varroa*'s movements were obtained in order to understand how *Varroa* initiates and subsequently succeeds to build a fecal accumulation (FA) on the cell wall in the posterior part of the brood cell during the prepupal stage and to show how it chooses a site for first oviposition. Since tracks leading to defecation start once *Varroa* climbs onto the cell wall after feeding, records were obtained by simply transcribing the position of the mites' mouthparts onto plastic sheets on the video screen as it walked on the cell wall. Thus, when the mite pivots the resulting track is circular. The hemispheric view of the cell wall was rendered planar without correction, resulting in most distortion at the lateral borders. Analysis of the different track types was made by plotting coordinates of points at which the tangent to the track was parallel to the long axis of the cell. The difference between two points, termed an interreversal leg (IRL), is then ascribed a Δx and Δy value, corresponding to, respectively, displacement perpendicular to and parallel to the long axis of the cell. Since tracks in two different cell sizes were analyzed, the cell diameter was ascribed 100 units, and Δy values were calculated with reference to the same scale, which thus can exceed 100 units. For each track, medians (to exclude outliers) were calculated for the first and last five Δx values and means were calculated for the first and last six Δy values (normally distributed); tracks with fewer than eight IRLs were excluded. The Wilcoxon matched-pair signed rank test was used to compare the start and end of tracks, and the Mann-Whitney *U*-test to compare values obtained for different track types.

Role of geotaxis in feces deposition. To test for a role of negative geotaxis in feces deposition, 102 *Varroa* mites were transferred from natural cells at the end of spinning (just prior to when they are normally predisposed to form the FA at 30 h post-capping, hpc) to non-infested artificial worker cells containing a prepupa at 60 hpc. During this period the prepupa adheres to the bottom of the cell permitting us to rotate the test cells ($n = 54$) 180° in the longitudinal axis so that the prepupa hung down. Control cells ($n = 48$) were not rotated. After introducing the females all cells were closed again with the original cap and held in the incubator. The position of all feces in the cell was noted after 24 h.

Ability of protonymphs to survive on their own. A few hours before pupation infested drone cells contain the first protonymph and the second egg (Fig. 1). Since the bee rids itself of the prepupal cuticle at pupation, we set out to determine if the protonymphs are capable of surviving on the as yet unpierced pupa on their own. Artificial drone cells were uncapped shortly before pupation and *Varroa* mothers within were retrieved with fine forceps. Control cells were only opened. All cells were closed again with the natural cap and maintained in a beehive protected by a metal mesh to prevent opening by worker bees. The cells were opened 4 days later to examine the state of offspring present at manipulation.

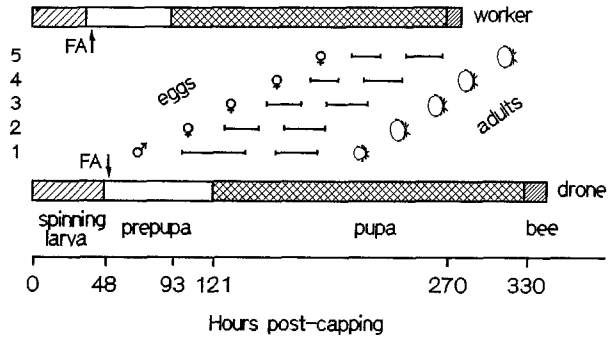

Fig. 1. Oviposition by *Varroa* and development of progeny from cell capping until bee emergence within artificial worker and drone honeybee cells. *Horizontal bars* represent mobile proto- and deutonymphs, and intervening blanks immobile stages (i.e., eggs and pharate nymphs); pooled data from single and multiinfested worker and drone cells. The fecal accumulation (FA) appears in both cell types only after the bee has stretched out as a prepupa. Note that two eggs are laid during the prepupal stage in drone cells but only one in worker cells. Durations of development for the different life-stages are given in Table 3. A sixth egg was laid in a small percentage of drone cells

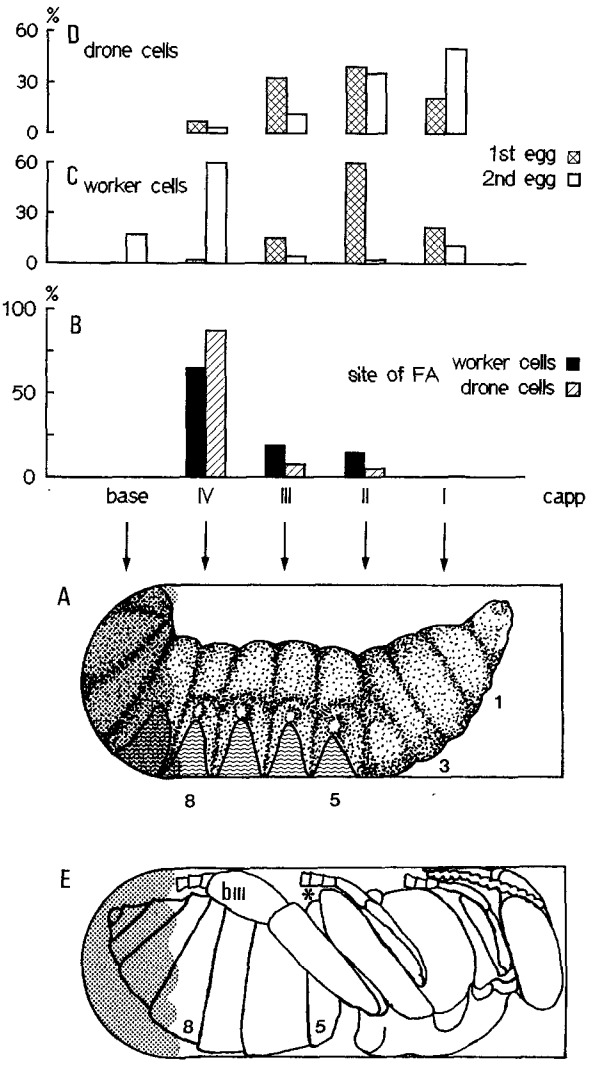

Fig. 2. **A** Lateral view of a worker prepupa with segments *numbered* from the first thoracic. For this study, the cell was arbitrarily divided into sections I to IV and cell base. *Wavy lines* drawn on abdominal segments represent contact with the cell wall. Bee excrement (*shaded*) is always deposited at the cell base and only during spinning. **B** Frequency of the fecal accumulation (FA) in different sections of natural and artificial worker and drone cells containing only a single FA (data pooled). **C** and **D** Site of deposition of first and second eggs in worker and drone artificial cells respectively (pooled data from single and multiinfested cells). **E** Lateral view of a young drone pupa. *Asterisk* indicates the feeding site on the 5th segment; *bIII* is basitarsus of leg III in its natural position. Thorax plus leg pair II form a barrier separating the cell into an anterior (i.e., towards the cap) and posterior part. The only opening in this barrier is between the tarsi of legs II

Results

From cell capping to pupation

After capping, the bee larva begins to make regular somersaults, which last 39 ± 8.4 min ($n = 40$), and feeds in bouts on the jelly for the first 5 h. After this it starts to spin the cocoon against the cell wall. The *Varroa* female climbs from the jelly onto the bee as it feeds, and almost immediately feeds itself for the first time. The mite evidently avoids being trapped by "piggy-backing" alternately on the larval extremities and ventral side, surfaces which come least in contact with the cell wall. *Varroa* thus stays almost exclusively on the spinning larva, which moves all the time, but the mite succeeds in feeding for ca 207 ± 125 s ($n = 12$) every 1.6 h. *Varroa* defecates for the first time at some 5 hpc. During this early phase of bee development the rather transparent feces are deposited randomly by *Varroa* on the cell wall and are subsequently covered by the cocoon. By 24 hpc *Varroa* has already defecated 11 ± 3.5 times ($n = 9$ cells).

The cocoon is spun by 33–36 hpc in worker and by 48–52 hpc in drone cells (Fig. 1). The bee prepupa then stretches out on its back with its head towards the cell cap leaving most free space inthe upper half of the cell (Fig. 2A), and remains so until pupation. At the early prepupal stage the mite already spends $46 \pm 27\%$ ($n = 12$ worker cells) of the time in section IV of the cell. The mite demonstrates a fervent interest in the anal zone of the bee, regularly probing it with up-down movements of the head. In drone cells, the bee's anal zone is so attractive to *Varroa* that some get trapped behind the terminal abdominal segments until pupation.

Fecal accumulation

At the beginning of the prepupal stage, the *Varroa* female shows a preference for defecation near the cell apex of section IV, corresponding to the position of basitarsus III of the pupa (Fig. 2B, 2E). At this period, the feces become milky. The process by which *Varroa* builds the fecal accumulation (FA) on the cell wall is best understood by dividing the tracks leading to its formation into the following classes: (a) *before FA formation* when no feces are as yet deposited on the new cocoon; (b) *onset of FA formation* when a few feces have already been deposited near one another; (c) *tracks leading directly to the FA* after it is well formed, i.e., when *Varroa* climbs onto the cell wall in its immediate vicinity; and (d) *rejoining the FA* in the

Table 1. Lateral (Δx) and longitudinal (Δy) displacement of *Varroa* mothers leading to defecations and first oviposition on the cell wall of brood cells of the prepupal stage of bee development (mean ± SD per track). The start and end of the same track type was compared (horizontally) by the Wilcoxon matched pair test. Comparisons (vertically) between values for different track types were made with the Mann-Whitney *U*-test. Values within columns followed by different letters are significantly different at the 5% level. FA, fecal accumulation

Track type	Displacement in the lateral axis						Displacement in the longitudinal axis					
	n	Track		Comparisons			Track		Comparisons			
		Start [units per Δx]	End	Start-end	Start-start	End-end	Start [units per Δy]	End	Start-end	Start-start	End-end
				Wilcoxon	Mann-Whitney				Wilcoxon	Mann-Whitney	
Before FA formation	6	80 ± 12.0	82.7 ± 11.0	n.s.	a	a	1.7 ± 1.9	0.5 ± 1.6	n.s.	ab	a
Begin FA formation	11	68 ± 20.0	30.5 ± 8.0	<0.001	a	b	0.1 ± 1.4	0.5 ± 2.2	n.s.	a	a
Direct to FA	8	30 ± 7.8	21.1 ± 2.7	<0.05	b	c	0.5 ± 2.0	0.1 ± 0.7	n.s.	a	a
Rejoins FA from anterior	15	36 ± 13.4	29.5 ± 8.0	<0.05	b	b	4.5 ± 3.2	1.4 ± 2.0	<0.05	b	a
Before laying	9	51 ± 12.4	51.6 ± 24.3	n.s.	c	d	−6.4 ± 5.5	−0.5 ± 3.8	<0.05	c	a

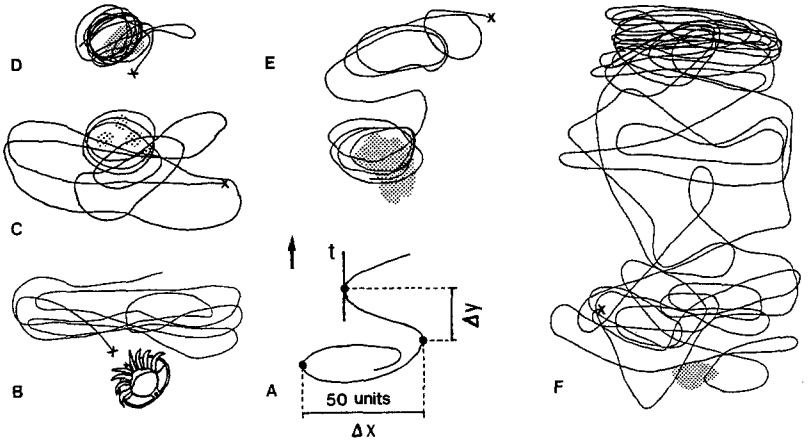

Fig. 3A–F. Tracks made by *Varroa* on the cell wall prior to defecation (B–E), and before oviposition (F) in artificial cells. *Shaded zones* represent *Varroa*'s feces on the cell wall, *x* the start of each track leading to defecation once *Varroa* regains the cell wall from the bee, and the *arrow* indicates the longitudinal axis of the cell towards the cell cap. **A** Difference between two points (●) on the track at which tangent (*t*) is parallel to the longitudinal axis of the cell (*arrow*) is ascribed a Δx and a Δy value corresponding, respectively, to displacement perpendicular and parallel to the long axis of the cell. **B** When no feces are present on the newly spun cocoon the mite climbs onto the cell wall from the anal zone and regularly recrosses the cell before defecating at the cell apex. **C** In a cell where four feces have already been deposited near one another the mite circles on the previously deposited feces. **D** The mite normally regains the cell wall from the bee via the borders of section IV during the prepupal stage such that the fecal accumulation (FA) is encountered almost immediately, and the mite pivots. **E** When *Varroa* regains the roof of the cell anterior to the FA it circles along one side of the cell before orienting to the FA. **F** Track leading from the FA to the first oviposition site anterior in the cell

posterior part of the cell after *Varroa* has climbed onto the cell wall anteriorly. The track types c and d are made when the FA is well formed and were therefore studied at >70 hpc.

The following analyses show characteristics of the tracks which lead the mite to the chosen defecation site (Table 1, Fig. 3). *Before FA formation* (Fig. 3B): within the newly spun cocoon, the mite mostly climbs (i.e., upturned) onto the cell wall from the anal zone and recrosses the lateral axis of the cell 8 ± 4 times in 28 ± 13 s, $n = 12$ tracks (80 units Δx^{-1} at outset versus 83 units Δx^{-1} at end for $n = 6$ tracks, n.s.) without leaving section IV (1.7 units Δy^{-1} at outset versus 0.5 units Δy^{-1} at end, n.s). Suddenly, the mite stops at the cell apex to defecate and then moves 0.5–1 mm away. *Onset of FA formation* (Fig. 3C): In cells where a few feces have already been deposited near one another in section IV, the number of times *Varroa* recrosses the cell roof before defecation is somewhat reduced (mean 5.4 ± 2.3 crossings in 31 ± 8.3 s, $n = 12$ tracks, n.s. compared to previous type), for once the mite encounters a previously deposited feces it turns 180° and successive IRLs grow shorter until the mite pivots on the previously deposited feces to defecate (68 units Δx^{-1} at outset versus 30.5 units Δx^{-1} at end, $P < 0.001$, $n = 11$ tracks). Such pivoting prior to defecation eventually leads to the construction of a compact FA since all feces are deposited near one another (Figs. 3C–D and 4). *Tracks leading directly to the FA* (Fig. 3D): During the prepupal stage the mite normally regains the cell wall from the bee via the borders of sec-

Table 2. Number and location of fecal accumulations (FA) in natural and artificial cells infested by a single *versus* several *Varroa* females. Sections refer to parts of the cell (Fig. 2) where single FAs were located: contingency test for randomness by Pearson χ^2. This test is not applicable when more than one-fifth of data sets have sparse values (<5)

Type of cell	Infestation level	Number of FA			Sections				Test for randomness
		One FA	>One FA	Fisher exact test	I	II	III	IV	
Artificial drone	uniinfested	88	13	$P>0.4$	5	5	10	68	$\chi^2 = 49.4$, df = 3, $P<0.0001$
	multiinfested	34	8		3	0	5	26	Test not applicable
Natural drone	uniinfested	34	10	$P>0.2$	0	1	4	29	Test not applicable
	multiinfested	51	8		0	0	9	42	$\chi^2 = 42$, df = 3, $P<0.0001$
Artificial worker	uniinfested	138	21	$P>0.7$	3	19	18	98	$\chi^2 = 66.9$, df = 3, $P<0.0001$
	multiinfested	27	5		0	1	2	24	Test not applicable
Natural worker	uniinfested	126	15	$P>0.08$	1	6	29	89	$\chi^2 = 73.9$, df = 3, $P<0.0001$
	multiinfested	11	4		0	0	1	10	Test not applicable
Total		509	84		12	32	78	386	

tion IV (81% of 312 observations in drone and 47% of 76 observations in worker cells) such that the FA is encountered almost immediately. In this case the mite pivots on the FA (30 units Δx^{-1} at start and 21 units Δx^{-1} at end, $P<0.05$, $n = 8$ tracks). *Rejoining the FA* (Fig. 3E): When *Varroa* regains the roof of the cell anterior to the FA the return journey to the feces typically involves circling behavior (i.e., short IRLs, 36 units Δx^{-1}, $n = 15$ tracks) unilaterally along the roof with a net displacement to section IV (4.5 units Δy^{-1} at outset versus 1.4 units Δy^{-1} at end, $P<0.05$) where it pivots on the FA (29.5 units Δx^{-1}, $P<0.05$ by comparison with the start) before defecation. For all tracks analyzed *Varroa* shows a significant tendency to turn repetitively in the same direction once it has started to turn. This tendency increases with feces concentration (for all tracks types $P<0.001$, $17.8<\chi^2<95$, $df = 1$).

Comparison between the different track types shows a significant decrease in lateral displacement both at the start and end of tracks as the feces is concentrated (Table 1); *Varroa* does not displace itself in the longitudinal axis of the cell during FA formation. According to where a particular mite habitually climbs onto the cell wall to defecate, the FA is sometimes placed in more anterior sections of the cell, but here also without any longitudinal displacement for cell apex location. With time, *Varroa* shows a behavior typical of that normally performed on the FA once it regains the cell wall, as shown by the short lateral displacement (35.5 units Δx^{-1}) which are observed immediately after it climbs onto the cell wall anterior to the FA. Even here, *Varroa* avoids depositing feces outside the FA towards which it eventually orients.

Only one FA was found in 509 of 620 artificial and natural worker and drone cells, and the FAs are placed preferentially in section IV in all types of cells (Fig. 2B, Table 2). The preference for section IV over other sections is the same in natural and in artificial cells ($P>0.8$ in worker cells; $P>0.2$ in drone cells; two-tailed Fisher exact test). In the hexagonal natural cells, feces are found at one or both sides of the apex but we consider it as one FA here for purposes of comparison. No FA was found in only 4.3% of cells, whereas two FAs were found in 13.5% of all cells observed (Table 2).

From 44 to 48 hpc in worker cells a pronounced FA has been formed. From here on *Varroa* spends more time holding station on it (from 19±27% at 48 hpc to 79±20% at 90 hpc) while regularly undertaking half-circle movements on the accumulation. These regular displacements lead *Varroa* across the FA, but the mite stops at the opposite border holding its mouthparts outside the FA. Subsequently all behavior of *Varroa* appears to be with reference to the FA and progressively the mite demonstrates the following behavioral routine: once hungry, *Varroa* descends onto the bee and accesses the feeding site. After feeding, the mite soon returns to the cell wall where it stops on or near the FA, usually to defecate. The duration of successive journeys between these two sites grows shorter with time.

The position of the FA near the cell apex indicates that *Varroa* may be displaying negative geotaxis for defecation. After 24 h in upturned cells, 87% of *Varroa* had formed the FA on the cell wall, 43% of these at the usual site on the floor of the upturned cell in section IV, and 35% in section I over the head of the prepupa. Only 3.7% of FAs were found on the prepupa. In unturned controls, where 81% constructed an FA, 51% of these were at the usual site in section IV and none behind the head of the prepupa ($P<0.001$, $\chi^2 = 17.8$, $df = 3$, compared to test).

Oviposition

Although *Varroa* has access to all parts of the brood cell at the prepupal stage it uses specific sites for egg laying. Tracks leading to first oviposition at 70 hpc (Fig. 3F, Table 1) involves heading away from the FA, recrossing the cell (5.8±5 crossings; 51 units Δx^{-1}, $n = 8$ tracks), and fast displacement away from section IV (6.4 units

Δy^{-1}) to forward sections of the cell where it undertakes vigorous criss-crossing of the cell (23 ± 19 crossings, $P < 0.05$; 52 units Δx^{-1}, n.s., $n = 8$ tracks), without displacement in the longitudinal axis (0.5 units Δy^{-1}), before stopping to oviposit at the cell apex. The whole track lasts 5.5 ± 4 min ($n = 30$). During this time *Varroa* probes the substrate with its mouthparts at each step, resulting in a characteristic jerky walk.

Once at the oviposition site, *Varroa* stops at the apex of the cell but continues to repeatedly sample the substrate with leg pair I for 1 ± 0.5 min ($n = 32$). As the egg emerges from the genital opening the first two pairs of legs bear it and sustain it against the cell wall. After some 10 ± 3.7 min ($n = 25$), *Varroa* removes her legs one at a time from the egg, leaving it slowly some 23 ± 5.7 min ($n = 31$) after laying. The reason why *Varroa* shows so much care in placing her eggs becomes clear when one considers that the protonymph within always has its legs oriented to the substrate, allowing it to walk away at hatching: protonymphs in disturbed eggs do not succeed in leaving the eggshell ($n > 15$).

The positions of eggs laid during the prepupal stage by the mite are described here with reference to the lateral and longitudinal axes of the cell. In artificial cells, 68% of eggs ($n = 238$) were placed at the cell apex. In natural cells, 63% of eggs ($n = 49$) were likewise placed in the apex angle and 27% in the upper lateral angles of these hexagonal cells. Thus, the upper angles of natural cells are employed for oviposition in 90% of cases, a mechanical guide evidently absent in our artificial cells. Furthermore, the first egg is laid preferentially in section II of worker cells ($P < 0.0001$, $df = 3$, $\chi^2 = 37$ and 40, respectively, for 70 natural and 107 artificial cells; test for randomness). The section of the cell in which the first egg is laid in natural and artificial worker cells is the same ($P > 0.25$, $\chi^2 = 3.9$; $df = 3$). In artificial drone cells, the second egg ($n = 61$) is laid before pupation (Fig. 1) and placed preferentially anterior to the first ($n = 104$) ($P < 0.001$, $\chi^2 = 17.5$, $df = 3$), but neither egg is placed at random ($P < 0.01$, $df = 3$, $\chi^2 = 15.5$ and 22 for first and second egg in test for randomness) (Fig. 2C,D). Most eggs laid after pupation in both cell types are placed in section IV on the border of the bee excrement.

From pupation to bee emergence

Varroa mothers reactions to bee pupation

A major change in the free space available for *Varroa* occurs when the prepupa moults into the pupa (Figs. 2A,E and 4). During the bee's movements, lasting some 30 min, to extend appendages and deposit the exuvium at the base of the cell, *Varroa* is frequently pushed off the FA but quickly returns. In 91% of pupated cells ($n = 168$) *Varroa* is located in the posterior part of the cell. Immediately after pupation *Varroa* engages in "leg-pushing" resulting in the displacement of one or both of the pupa's third legs (legs were displaced in 124 of 215 natural cells and in 131 of 206 artificial cells), thus enlarging the free space around the FA. This behavior, which takes up 67 ± 14% of the time prior to the first feeding on the pupa, disappears completely some 5–6 h later. In multiinfested cells, females were seen pushing simultaneously, but in an uncoordinated way. In all uninfested cells ($n > 1000$) the tarsi III are united on the ventral side of abdomen. In artificial and natural infested cells examined ($n = 421$), pupae present three different positions of their third legs: leg pair III unmaneuvered by *Varroa* (47 and 35% respectively, of drone and worker pupae), leg pair III separated and moved to the sides of the abdomen (27 and 42%, respectively, Fig. 4), and cells with one leg III pushed forward so that more space is free on one side of the pupal abdomen (27 and 24%, respectively). The proportion of pupae presenting these three-third leg positions is the same in natural and artificial infested cells ($P > 0.17$, $\chi^2 = 3.4$, $df = 2$, $n = 259$ in worker; $P > 0.9$, $\chi^2 = 0.2$, $df = 2$, $n = 162$ in drone pupae).

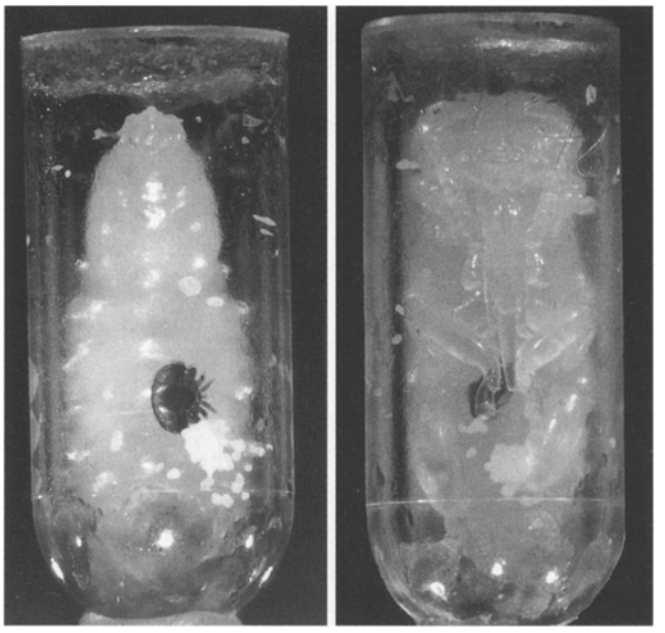

Fig. 4. *Varroa* mite parasitizing honeybee brood in artificial cells, prepupa (*left*) and young pupa (*right*); bee pupation occurs within 30 min, causing a radical change in the free space available for *Varroa*. *Left* The parasite begins to construct a fecal accumulation (*white mass* on cell wall of both cells) even at the prepupal stage. The *first egg* is laid on the cell wall at the level of the constriction between the abdominal and thoracic segments of the prepupa. *Right* *Varroa* has moved the pupal legs III laterally for access to the ventral side of segment 5, the preferred feeding site (occupied)

Eggs and developmental stages

Despite the difference in host development between drone and worker cells, oviposition of the single haploid egg (i.e., male) begins at the same time in both cell types, at 71.8 ± 2.7 hpc ($n = 21$) and 69.5 ± 2.6 hpc ($n = 12$), respectively (Table 3). Diploid eggs (females) are laid thereafter at 29.7 ± 1.1 h intervals ($n = 43$) and the fifth egg is laid at 190.3 ± 7.7 hpc ($n = 6$ worker cells). In some drone cells a sixth egg is laid. Successive newly moulted

Table 3. Oviposition and duration of development of successive *Varroa* descendants (means ± SD; h). Hpc, hours post-capping

	First descendant		Second descendant		Third descendant		Fourth descendant		Fifth descendant	
	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*
Oviposition (hpc)	70.9 ± 3.2	34	101.7 ± 2.9	23	130.3 ± 3.3	8	161.2 ± 8.5	5	190.3 ± 7.7	6
Hours since previous laying			30.2 ± 1.1	21	29.3 ± 0.8	7	29.0 ± 0.9	8	29.5 ± 1.0	7
Egg[1]	29.9 ± 1.4	28	27.4 ± 1.5	8	26.8 ± 2.0	6	25.4 ± 2.2	5	24.0 ± 1.9	6
Mobile protonymph[2]	42.5 ± 5.7	16	22.9 ± 1.7	8	23.2 ± 1.9	7	18.7 ± 2.1	7	19.3 ± 3.9	9
Pharate protonymph[3]	20.0 ± 1.1	9	17.0 ± 1.5	7	16.9 ± 1.7	6	17.2 ± 2.1	8	17.2 ± 1.9	6
Mobile deutonymph[2]	28.1 ± 2.4	9	27.3 ± 3.0	7	27.7 ± 2.7	6	25.7 ± 1.2	5	24.6 ± 1.7	3
Pharate deutonymph[3]	35.1 ± 2.4	9	48.0 ± 2.2	7	47.1 ± 2.2	5	49.0 –	1	– –	–

Data from single and multiinfested worker and drone cells (pooled); [1] from oviposition until onset of hatch, [2] from onset of moult or hatch until immobilization, [3] from immobilization until onset of moult

adult females therefore have decreasing lengths of time within the cell for mating and maturation before emergence of the bee (Figs. 1 and 5). Two eggs are laid during the prepupal stage in drone cells, such that the first protonymph is mobile at bee pupation.

Feeding pattern of *Varroa* mother

The feeding pattern of the mother varies greatly during the course of the reproduction cycle (Fig. 5). On the prepupa she feeds often (1.1 ± 0.36 bouts h^{-1}, $n = 13$ individuals) but for short periods (161 ± 109 s per bout, $n = 425$) and there is still no preference for a particular segment as a feeding site. Although the *Varroa* female often changes location she always feeds on the abdominal pleural pads (523 of 525 observations). On the contrary, during the pupal stage she feeds less often (0.34 ± 0.04 bouts h^{-1}, $n = 10$ females) but longer than on the prepupa (495 ± 254 s per bout, $n = 127$; $P < 0.0001$, Student *t*-test), and always at the same site (see below). Despite these differences the mean duration of feeding per unit time is exactly the same (173 ± 46 and 173 ± 51 s h^{-1}, $n = 13$ and 10 individuals for prepupal and pupal stages, respectively; $P < 1.0$, Student *t*-test). These periods correspond to oogenesis in *Varroa*, considered here to occur during the prepupal and pupal stages from 36 until 180 hpc. The 20 h following pupation when *Varroa* feeds for prolonged periods were not included in these calculations since during this period the feeding bouts are very long, decreasing exponentially to stabilise some 15 h later (Fig. 5). Between laying the last egg and imaginal ecdysis, the mother feeds less often (0.12 ± 0.04 bouts h^{-1}, $n = 8$ individuals) and for longer (693 ± 259 s per bout, $n = 56$) but only for 84 ± 18 s h^{-1} ($n = 8$ individuals; $P < 0.001$, Student *t*-test).

Establishment and importance of the feeding site

During the "leg-pushing" period the female feeds for the first time at 132 ± 40 min ($n = 15$) after onset of pupation. By contrast with the short feeding bouts of 2.7 ± 2 min ($n = 525$) on the prepupa, the first feed by the adult female on the pupa lasts 60 ± 25 min ($n = 15$) (Fig. 5). In cases where *Varroa* succeeds in prising the third pair of basitarsi apart, 39 of 50 feeding sites were established ventrally on abdominal segment 5 (Fig. 4). Even when *Varroa* does not succeed in pushing the legs apart, some (12 of 63) still reach the ventral zone of segment 5 to feed. In all other cases, *Varroa* preferentially feeds laterally on one of the abdominal segments 6–8. This single feeding site per pupa is used thereafter by adults and progeny (including adults of both sexes) in single and multiinfested cells. This is true in 86% of cases where the 5th segment was the choice ($n = 36$ cells for at least 48 h of observation), but when another feeding site was chosen only 50% used the same one throughout the observation period ($n = 30$ cells). In the latter case, mites either moved from a feeding site which had become melanized or fed simultaneously from two sites. Apparently an advantage stems from use of segment 5, since feeding other than on this segment occasionally resulted in bee hemorrhage and then drowning of *Varroa* progeny was observed ($n = 6$ of 67 cells).

Is the investment by the mother in establishing the single feeding site critical to her descendants? When the mother was experimentally retrieved from cells before her first feed on the pupa, all protonymphs ($n = 93$) were recovered dead 4 days later in 33 cells with mothers removed, whereas 57 of a total of 85 offspring at opening developed normally in 26 control cells. Despite modifications of its chelicerae as spermadactyls, the male feeds regularly thanks to the prepared feeding site (Fig. 5). Ingestion of hemolymph by males was confirmed by the fact that they continued to defecate even several days after molting. As *Varroa* oviposits in the anterior part of the cell during the prepupal stage in both drone and worker cells (Figs. 1 and 2) one individual hatches following pupation in front of leg pair II which form a barrier across the cell (Figs. 2E and 4). These protonymphs are hyperactive until accessing sections III + IV of the cell via the only passage between tarsi II (33 succeeded out of 51 observed). Those which remain forward of leg pair II die after some 20 h for want of access to the feeding site.

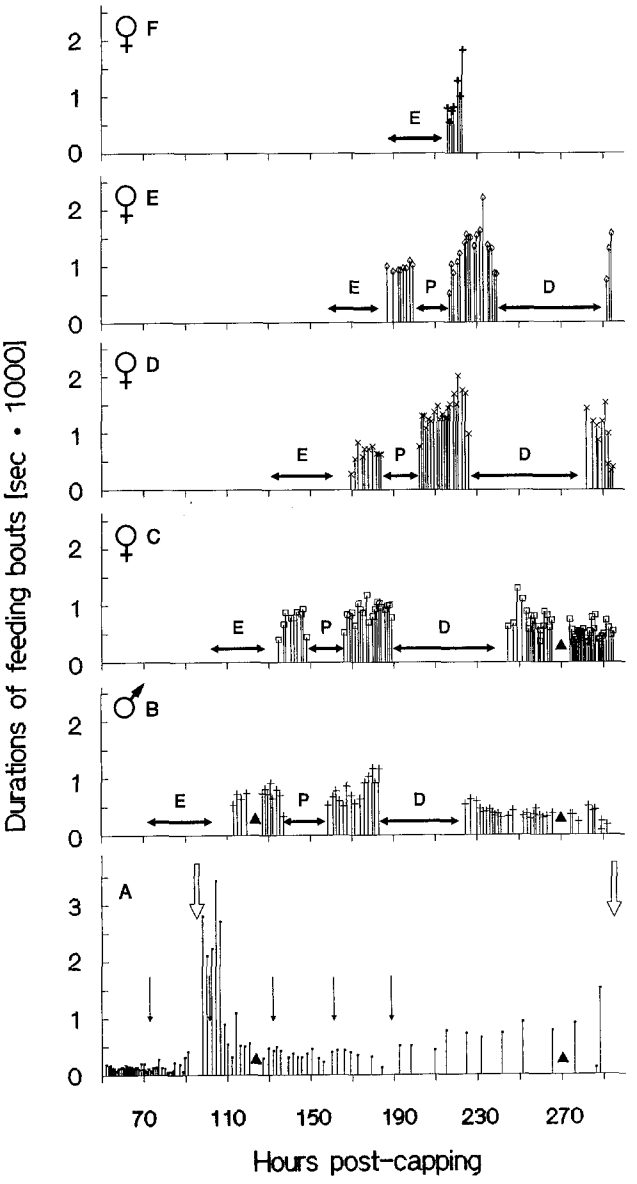

Fig. 5A–F. Incidence and duration of feeding bouts by all mites in a singly infested artificial worker cell from 50 h post-capping (hpc) until emergence of the bee. **A** *Varroa* mother feeding pattern. The *open arrows* indicate pupation (96 hpc) and imaginal ecdysis (294 hpc) of the bee, and the five *thin arrows* indicate ovipositions. **B** The feeding pattern of the single male descendant, and **C–F** feeding pattern of the four successive female descendants. *Horizontal arrows* represent immobile development stages (*E*, egg from oviposition until onset of the hatch; *P* and *D*, pharate proto- and deutonymph from immobilization until onset of ecdysis). The fifth descendant did not survive. Missing data are indicated by ▲. All feeding took place at a single feeding site on the pupa at the ventral side of the 5th segment as in Fig. 4

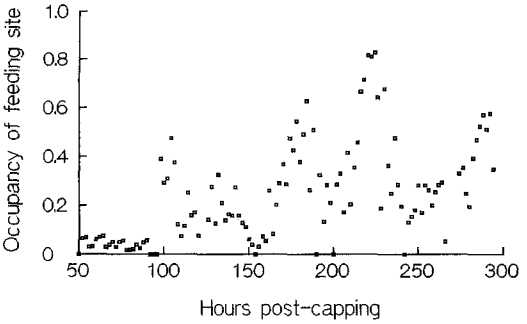

Fig. 6. Occupancy of the single feeding site in the worker cell represented in Fig. 5, as a proportion of 2-h observation intervals

Competition at the feeding site

Concentration of the activity of all mites to two zones within the cell, i.e., at the FA and feeding site, results in frequent interactions between them. On the FA mites are often pushed off but halt nearby before they return to it. Such disturbance on the FA is probably not prejudicial to the development of nymphs. In contrast, occupancy of the single feeding site (Fig. 6) is cyclic due to regular reappearance of mobile instars in the cell, and negative consequences could be expected here if competitive interactions arise between individuals. To investigate this, we followed two cells (one singly and one doubly infested) during a 147-h period when high numbers of mobile stages occur. To determine the length of time mites showing appetitive behavior had to "wait" because the single feeding site was already occupied by another individual, the time from encounter with the previously feeding mite until the site was freed for the searching mite was measured. The searching mite was occasionally obliged to "wait" even longer due to opportunistic occupancy of the site by yet another individual.

"Waiting" individuals not only engage themselves in active search for a feeding site but were often observed to push the feeding mite and sometimes even tried to slip under it. Others just stay immobile behind the feeding mite or return to the FA. All instars were obliged to "wait" at least once at the feeding site even in singly infested cells. Although frequency of waiting does not differ statistically between instars, protonymphs must wait significantly longer than all other instars (Table 4). This difference is because protonymphs have difficulty in localizing the feeding site (Fig. 7), to the point of even permitting an additional mite to occupy it first. Furthermore, we observed that adult mites regularly displaced a feeding protonymph: 32 out of 73 protonymphs were observed to desert the feeding site just after being pushed by a newly arriving adult or deutonymph. The frequency of interrupted meals decreases in bigger instars (in deutonymphs, $P<0.0001$; males, $P<0.05$; and *Varroa* mothers, $P<0.0001$, comparison with protonymphs, Fisher exact tests). The duration of the interrupted feeding bouts is shorter than uninterrupted ones for almost all instars (but significant only for the deutonymphs). These observations clearly indicate that competition arises between individuals at the feeding site and that protonymphs are most disadvantaged.

Multiinfested cells

What happens when two or more females invade a cell simultaneously? Do they form the same FA, show the

Table 4. Interactions at the single feeding site for *Varroa* developmental stages and adults. The Fisher exact test was employed to make pairwise comparisons (horizontal) of "waiting" and interruption frequencies between instars. The Mann-Whitney *U*-test was used to compare differences in "waiting" durations between instars (horizontal), and feeding bout durations of interrupted as against uninterrupted feedings (vertical). Different letters indicate significance at the 5% level or lower

	Protonymphs	Deutonymphs		Adults		
		female	male	male	daughter	mother
Observed feeding bouts	73	98	11	21	29	32
Observed "waiting" for feeding site	35	35	7	8	11	13
Fisher exact test	a	a	a	a	a	a
Duration of "wait" [min.]	33±26	10±7	13±10	16±10	13±8	9±8
Mann-Whitney U-test	a	b	b	b	b	b
P values for comparison with protonymphs		<0.0001	<0.01	<0.05	<0.001	<0.0001
Number of interrupted feedings	32	14	4	4	7	2
Fisher exact test	a	cb	ab	bc	abc	c
Feeding duration: uninterrupted [sec]	836±320	1120±320	914±230	439±108	653±342	585±304
Feeding duration: interrupted [sec]	788±422	873±314	798±325	381±121	670±345	268±216
Mann-Whitney U-test comparison between interrupted & uninterrupted feeds	n. s.	$P<0.02$	n. s.	n. s.	n. s.	n. s.

same oviposition site preferences and occupy the same feeding site? The proportion of all cells, i.e., single and multiinfested, worker and drone, natural and artificial cells, where only a single FA is formed varies between 73 and 89%. Comparisons between single and multiinfested cells of all types do not show any significant difference (overall $P>0.08$, Fisher exact test, Table 2). For all types of single and multiinfested cells, the single FA is situated preferentially in section IV. Eggs laid during the prepupal stage are placed preferentially in the anterior part of both single and multiinfested cells. However, these eggs are deposited on average more anteriorly in multiinfested cells than in singly infested ones ($P<0.01$; $\chi^2 = 15.1$, $df = 3$ in artificial drone cells; test not applicable to other cell types due to low *n*). During the pupal stage a single feeding site was used in 27 of 43 single infested cells and in 17 of 23 multiinfested cells ($P>0.4$, Fisher exact test). The latter cells were infested by either two or three females, but we have no information for cells with higher rates of infestation. Despite the dense population of *Varroa* mothers and progeny in certain cells, we never observed cannibalism.

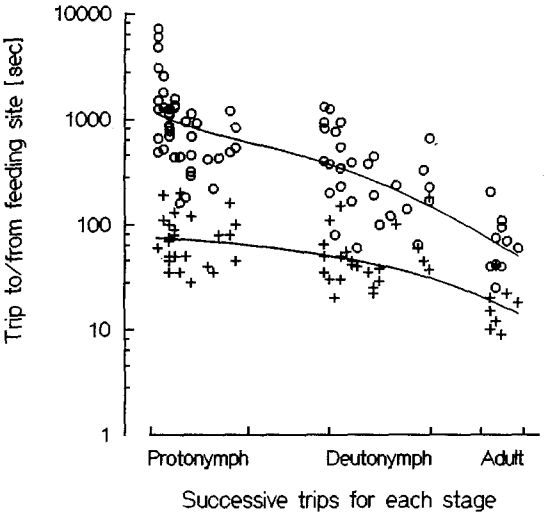

Fig. 7. Duration of successive trips from the fecal accumulation on the cell wall to the feeding site on the bee (○), and vice versa (+), made by developmental stages and adult *Varroa*. Trend lines were calculated with the distance-weighted least-squares method (Wilkinson 1990). Trip durations represent time from mobilization on the FA until onset of sucking at the feeding site, and from removal of mouth parts from the bee to arrival on the fecal accumulation. Only trips made when the feeding site was accessible were retained, so values plotted correspond to the true search efficiency

Varroa's use of the structured living space

During the pupal stage the mite population in the cell increases with hatching of successive protonymphs so that at 240 hpc this leads to the presence of mother, adult son and daughter, and at least one mobile nymph. Except for a few hours at the beginning of the protonymphal stage, the behavior of all mobile instars is clearly dominated by a strong tendency to remain on the FA, with individuals leaving it only to feed on the pupa. The mites mostly follow the same path (62 of 106 observations) to cover the distance between these two sites. With time, the duration of successive return trips to and from the feeding site made within a given developmental stage shortens, and this is conserved after molting (Fig. 7). Since activity of the mobile instars is concentrated in the zones where the FA and the feeding site occur, a high proportion of deutonymphs move to the bottom of the cell behind the pupa to moult, where there is less risk of being disturbed by congeners. However, newly moulted individuals return to the FA almost immediately. Remaining on the FA evidently facilitates frequent rematings since first mating occurs within 14±10 min of arrival of newly moulted adult females on the FA ($n = 10$), and over 90% of all mating events observed ($n = 143$) occur on the FA. *Varroa* males simply await female arrival at the FA, crisscrossing it regularly from one side to the other but rarely moving away from it except to feed. A male was never seen to attempt to mate with nymphs, but sometimes

began the mating sequence with his mother only to quickly desist.

After imaginal ecdysis the adult bee is very active until emergence some 20 h later, so that normal activity of the *Varroa* family within the cell is totally disrupted.

Discussion

These results demonstrate some remarkable adaptations on the part of *Varroa* in parasitizing the developing bee within the brood cells. This starts with hiding in the jelly to avoid detection before capping of the cell (Ifantidis 1988). Following this the mite shows great ability in making the appropriate movements on the spinning larva to avoid being trapped. When the prepupal stage is reached, the infesting *Varroa* establishes a fecal accumulation (FA) at the apex of the cell near the preferred feeding site on the pupa and within the closed-off end of the brood cell. The mother and all mobile nymphs and young adults rendezvous on the FA and matings between mature descendants occur exclusively here. Since production of a maximum number of fertilized daughters is at a premium for *Varroa jacobsoni* as a parasite of honeybee brood cells, the time limit set between capping of the cell and bee emergence probably acted as a selective pressure for the development of specialized adaptations.

Behaviors leading to establishment of the fecal accumulation

Construction of the FA on the cell wall rather than on the bee obviously preempts its disappearance at pupation and its position on the cell roof is due to the fact that *Varroa* shows negative geotaxis when defecating. This was demonstrated in the upturned prepupal cells where a third of mites sought out the only available free space to defecate, i.e., on the roof of the cell behind the head of prepupa. Only a few *Varroa* defecated on the bee, despite the fact that it covered most of the roof. Other *Varroa* females did defecate on the vacant floor of these upturned cells, but mostly near the anal zone – the region for which the mite shows greatest affinity during the early prepupal stage. The combined effects of negative geotaxis, attraction to the anal zone and avoidance of the bee go some way to explain why *Varroa* shows a preference for defecating on the cell roof not far from the anus in natural brood cells.

Varroa undertakes frequent criss-crossing of the cell before stopping at or near the apex to defecate on the unsoiled wall of the cocoon. One must remember that in natural cells the apex forms an angle and probably can be more easily located. *Varroa* no longer undertakes cell crossings to the same extent before defecating once a few feces have been placed on the cocoon, but is arrested on the previously deposited ones and pivots before defecation. Placement of the FA at one or both sides of the apex angle, rather than placing of the feces into the angle, would suggest that *Varroa* also pivots before defecation in natural cells. Pivoting behavior is often seen in arthropods following their perception of a chemostimulant (Bell 1985; Rovner 1991; Royalty et al. 1993). Our results suggest that the female learns to recover the FA by circling once on the cell wall, but flexibility in the type of arc described is shown when the FA is not immediately encountered. This behavior evidently permits *Varroa* to systematically regain the FA, thus avoiding construction of another.

Significance of the fecal accumulation

Due to the tenacity of *Varroa* in maintaining contact with the FA during bee pupation, the mite finds itself posterior to the obstacle created by the bee's leg pair II across the cell after the pupation. The only passage to the anterior is between the tarsi of leg pair II, but this is not sufficiently wide to allow *Varroa* adults to pass. Use of the posterior region of the cell by *Varroa* and concentration of its feces at one site there could reduce any odorous emissions, a factor which may be of significance in the context of reducing detection by workers of the original Asian host *A. cerana*. Phytoseiid predators are known to be attracted by prey silk and associated feces of Tetranychidae (Hislop and Prokopy 1981; Dong and Chant 1986), and *A. cerana* workers frequently open infested brood (Peng et al. 1987a,b; Rath and Drescher 1990). It is worth noting that the cap of *A. cerana* drone brood is perforated (diameter = 0.3 mm) (Hänel and Ruttner 1985; Rath 1992), a factor which may facilitate detection of any mites living in the anterior part of the cell. By confining the family to the rear end of the cell, and by concentrating its feces in one place, *Varroa* has probably developed a tactic of reducing detection, at least when infestation densities are low.

Varroa re-establishes the behavior routine shown on the prepupa after establishing a feeding site on the pupa, i.e., holding station on the FA interrupted by trips to the bee to feed. Defecation followed immediately after feeding in most cases. The FA eventually functions as a rendezvous site for all the *Varroa* progeny in the cell, for very soon after the first meals on the bee, each protonymph finds its way to the FA. This serves to avoid both crowding of the feeding site and soiling of the bee with feces. Nymphs only leave the FA to feed and moult, so few *Varroa* stray off to other areas of the cell. The distance to the feeding site is minimal, and return trips from the FA to the feeding site made by the successive development stages shorten with time. Whether this is due to more efficient use of a chemically marked path or memorizing of the way is unclear. The net result is a reduced journey time. All this suggests that the rendezvouz by *Varroa*'s mobile stages on the FA serves as a great time and energy saver for the parasite in its race to maximize the number of fertilized offspring before bee emergence.

Since males do not survive outside brood cells, fertilization of the adult daughters within the cell is paramount. However, low male survival in the brood cell acts as a factor limiting population growth. The first of

the two eggs laid during the prepupal stage in drone cells is male and this protonymph is active at bee pupation. A proportion of these male protonymphs, and eggs in both cell types does not survive the bee's movements during pupation. This partially explains why only 60% of males were found to survive in singly infested cells (Fuchs and Langenbach 1989; Otten 1991). The probability of finding a male in multiinfested cells increases (Fuchs and Langenbach 1989; Donzé et al., in prep.). The male, which matures first, passes an extended period on the FA with his mother and attempts to mate with her but without success. Newly moulted adult daughters are fertilized within a few minutes of arrival on the FA. Contrary to what occurs in other mite species, where males seek out pharate females (Potter and Wrensch 1976), *Varroa* males simply await female arrival at the FA. Concentration of all females at the same site, as against random encounters between the sexes elsewhere in the brood cell, probably serves to augment the proportion of fertilized females. This can be suggested for two reasons. Newly moulted adult females are more attractive than previously mated ones as the male shows selective attention to them once they arrive on the FA, and females already on the FA are remated frequently (Donzé et al., in prep.).

Once the last egg has been laid, the *Varroa* mother feeds less, clearly indicating her lowered requirement for nutrients. She shows a progressive loss of interest in returning to the FA, and in some brood cells shrinking of the bee allows the mother to make her way to forward sections of the cell before bee emergence.

Special oviposition adaptations

Varroa lays its eggs on the roof in anterior sections of the cell at the prepupal stage. Evidently, laying eggs on the cell wall and not on the bee preserves them from being entrapped in the exuvium at bee pupation. Subsequent eggs are likewise laid on the roof of both worker and drone cells in the posterior sections of the cell, but never on the FA. Negative geotaxis would appear to influence oviposition as almost all eggs are found at the cell apex. During the prepupal stage, when eggs can be laid in anterior sections of the cell, the mite exhibits directed movements away from the FA along the cell roof until the preferred zone for oviposition is reached. She then undertakes numerous lateral crossings of the cell, just as prior to first defecations on the cocoon, in what is obviously an effort to locate the cell apex, for it is here that most eggs are located in natural cells. The high number of crossings observed in our round artificial cells before oviposition could be interpreted to arise from the difficulty of localising the apex. However, one should remember that natural cells are rounded following spinning of the several cocoons, in which case they no longer really differ from our artificial cells. This could explain the ability of the mite to localize the apex of our round cells. Eggs are deposited very meticulously by the mother to insure that the protonymph can walk away at hatching; disturbed eggs do not yield viable offspring.

One may ask what advantage accrues to *Varroa* from depositing its first eggs in forward sections of the cell, zones virtually cut off after pupation from sections III and IV where the *Varroa* family matures. Firstly, it distances eggs from potential disturbance by female activity on and around the FA, an activity which is heightened after pupation when females engage in creating a living space. Secondly, by being placed at the apex angle in forward section of the cell the eggs escape the unpredictable hind pushing movements of the pupal appendages, thus avoiding being pushed away with the exuvium to the base of the cell.

The single feeding site

It is of significance that *Varroa* never feeds on the thorax or head of the prepupa, thus avoiding possible damage to the developing appendages. The mother restricts her feeding to one site on the bee after pupation. This is remarkable in a number of respects. Firstly, the length of time invested by the mother on the feeding site suggests that opening of the wound on the pupa requires a high investment. The long period of some 60 min spent by the female at this task is probably not related to any physiological requirement of hers, since the meal is disproportionate to any undertaken before or after. It is more likely to be related to the incapacity of protonymphs to pierce the host. Mites do not feed from abandoned and melanized feeding sites. Since the female feeds regularly, a single feeding site located in the vicinity of the FA is maintained. This will evidently reduce the time invested in searching for a meal by all nymphal stages at one as against a number of feeding sites, some of which may not have been maintained.

Descendants who first reach adulthood are more capable of competing for the feeding site and consequently are probably endowed with a higher chance of survival. Another advantage of the fixed feeding site is that adult males can also feed despite modification of chelicerae as spermadactyls. This repudiates the belief that male *Varroa* do not feed. Feeding permits male *Varroa* to survive and produce sperm during the 5 days from imaginal ecdysis of the male to bee emergence, the period over which female progeny moult into adults.

Feeding on the pupa via the 5th segment, as occurs in the case of another insect parasitic mite (Baker 1991), appears to have a significance for *Varroa*. *Varroa* females also show a strong preference for the lateral intertergites III on the left side of the abdomen on adult bees (Delfinado-Baker et al. 1992). When the parasite cannot access the preferred site on the 5th segment of the pupa, it can feed from a number of sites on the abdomen, but the suitability of any single one of them is apparently shorter in duration. Furthermore, in such cells the risk of hemorrhaging of the bee is higher, and this can lead to drowning of the nymphs.

Behavior and space structuring

One of the most pronounced features of the *Varroa* mother's behavior is her ability to switch on appropriate be-

haviors required for specific situations within the brood cell. Although the brood cell can be viewed as a rather constant environment, the bee's development causes some dramatic changes in the space available for *Varroa* and, during specific events such as pupation, can render the brood cell pretty inhospitable for the parasite. Although certain behaviors of *Varroa* are strictly programmed, such as feeding and oviposition, the parasite shows some plasticity with others. The *Varroa* mother, for example, feeds regularly on the bee larva, prepupa and pupa except immediately after pupation. Feeding therefore appears to be directly linked to oogenesis, which begins shortly after capping of the cell (Akimov et al. 1990; Steiner 1992,1993) and to be induced by a host factor present only for a short period (Rosenkranz 1990; Beetsma and Zonneveld 1992). Oogenesis progresses independently of whether the mite feeds on worker or drone developmental stages, since oviposition follows the same time pattern and regularity in both types of cells. In contrast to this physiologically driven fixed type of behavior, *Varroa* demonstrates great plasticity in responding to the changes in the space available. The mite for example makes no attempt to build an FA until the bee has spun the cocoon and has stretched out as a prepupa. This occurs some 12 h earlier in worker than in drone cells. It is therefore the nature of the space available which serves as the cue for the mite to construct the FA, and not *Varroa*'s physiological age. Once the FA has been constructed, the mite becomes progressively rigid in its behavior routine. In fact it only leaves the FA to feed on the bee, faithfully returning to it after each feeding bout to defecate. Holding station in this manner on the FA serves its purpose during bee pupation, when the mite is often jostled by the pushing movements of the bee's legs. By holding ground on the FA, *Varroa* is sure of finding itself in the posterior part of the cell after pupation, the preferred zone for feeding and defecation.

The basitarsi of leg III frequently cover the region of the upper cell wall near the FA following pupation. At this point *Varroa* undertakes "leg-pushing" from its position on the FA in order to dislodge the basitarsi laterally, thus providing access to both the FA and the preferred feeding site on pupal segment 5, and simultaneously enlarges the living space available in the posterior of the cell. This energy-demanding engagement by *Varroa* with the legs of its host will persist for over 90 min before its first feed on the pupa, at a time when the parasite has not fed for over 3 h. *Varroa* fails to dislodge either one or both legs in some 40% of cells. In this case the parasite again manifests some behavioral plasticity and establishes a feeding site on one of the more accessible abdominal segments.

One of the consequences of the structured living space for the *Varroa* female and her progeny is a simplification of behavioral activity, which becomes rhythmic following the physiological requirements of the mites. This limits the consumption of energy by restricting activity of mobile individuals to a small part of the cell and simultaneously facilitates mating.

Group behavior in Varroa

The evolution of the sociality in arthropods is thought to have followed the subsocial (Wheeler 1926) and the parasocial routes (Lin and Michener 1972). The subsocial sequence is characterized by co-operation in the daughter generation which stays in the family nest. The evolution of subsociality follows a continuum in the development of its complexity from simple oviposition, through egg watching, to direct feeding of the maturing descendants (Eickwort 1981; Brandmayr 1992). The evolution of sociality is thus aided by relatedness between cohabitants (kin selection; Wilson 1975) and, in some cases of haplodiploid species, by inclusive fitness (Hamilton 1964). In the parasocial scenario, on the contrary, several adults of the same generation (related or not) form a common nest and care for offspring (Michener 1974). Although many arachnid species are known to present a large variety of social behaviors which probably evolved in the above mentioned ways (Buskirk 1981), only a few examples of primitive sociality are known for the Acarina (Treat 1958, 1975; Saitò 1983, 1986a,b). In these species the structure of the group is subsocial since it is formed by a fertile female, and young adult offspring remain in the nest.

Maintenance of a single feeding site on the bee by the Varroa female has consequences for survival of her offspring. This is shown by the incapacity of protonymphs to survive on their own on the pupa after removing the mother, or when confined to the anterior of the cell by the bee's legs after pupation. The feeding site has added advantages for adult males allowing them to feed all their life. Utilization of the FA as a rendezvous site has the double function of facilitating the localization of the feeding site nearby for offspring, and inhibits descendant dispersal. The latter has consequences for fertilization of daughters. Since the behavior of the mother no only serves herself, but also her offspring, we conclude that *Varroa* exercises parental care.

Since all individuals contribute to cell sanitation and avoid crowding of the feeding site this represents an aspect of group behavior. Despite the competition between individuals at the feeding site, the overall picture is one of mutualistic (co-operative) behavior permitting the highest number of descendants to survive, as against monopolization of the single feeding site by the biggest (oldest) one. Thus, despite the cost of each trip to the feeding site (i.e., about 1000 s for protonymphs), the benefit is represented by the accessibility of the maintained feeding site once the individual gets there. Establishment of a number of feeding sites would probably increase the risk of host mortality.

In multiinfested cells, which occur frequently with *Varroa* (Moosbeckhofer 1988; Fuchs and Langenbach 1989; Tewarson et al. 1992), construction of a single FA, and use of the same FA by several mites of the same generation, are relevant to the definition of parasociality (Lin and Michener 1972). One may therefore ask how facultative "parasocial" behavior arose or is indeed maintained, considering how *Varroa*'s communal habit within the brood cells is so rudely disrupted by emergence of the

bee. One must distinguish between innate and learned responses in any discussion on the factors which may contribute to this. Outside the brood cell (*in vitro*), *Varroa* is arrested on a flat surface by chemostimuli extractable from the feces and the bee's cuticle (Donzé and Rickli, unpublished). It is therefore plausible to suggest that *Varroa* also responds to chemostimuli under appropriate conditions within the brood cell i.e., to bee chemostimuli when hungry and to feces-associated stimuli when it wishes to defecate. Construction of the FA by the mother might therefore result from a need to maximize chemosensory input from feces at the preferred location on the roof. *Varroa* protonymphs, incapable of feeding on their own, emerge into an environment where a living space, feeding site and FA have all been prepared. The descendants are therefore preadapted from the outset for the communal use of resources. In addition, maturing individuals show a remarkable ability to learn the most efficient use of this structured space, as exemplified by the shorter duration of return trips to the feeding site from the FA made by successive development stages. Experiences in the early stages of life are known to profoundly affect the life-long behavior of a whole range of species including insects (Lewis and Tumlinson 1988; Hérard et al. 1988; Vet and Groenewold 1990; Tumlinson et al. 1993). So the communal habit and tolerance described here for *Varroa* mothers in multiinfested brood cells could well result from experiences, such as waiting at the feeding site and holding station on the FA, made with other family members during their own maturation.

Other acarine species known to practice group behavior all live in defined habitats and form structures similar to *Varroa* (Treat 1958, 1975; Saitò 1983, 1986a,b). We must therefore ask if use of "cavities" by Acari (as represented by the host tympanic organ, web cells or brood cell) has permitted or even required the development of these structuring behaviors. On the one hand, these concealed habitats protect the mites and provide a stable environment near a food source, but on the other hand they require the mites to avoid competition and fouling of the habitat. Furthermore, some predisposing factors may contribute to the development of the habits particular to *Varroa*. This includes the predictability of the environment in which *Varroa* reproduces and the low genetic variability of the species (Biasiolo 1992). The latter arises from the poor dispersal of the parasite between honeybee colonies (Sakofski et al. 1990), and from its peculiar mode of reproduction where the haploid male fertilizes his diploid sisters. However, we refrain from cataloguing *Varroa*'s group behavior within any particular "sociality" category as most of the behaviors observed within the confines of the brood cell can be described as being either innate responses of the species or as resulting from the parental care afforded to progeny.

Consequences for Varroa *as a parasite*

Although dispersion of progeny within the hive is assured due to the quantity and proximity of brood cells, the original host exerts strong pressure on parasite population growth through grooming and elimination of infested brood cells (Peng et al. 1987a,b; Rath and Drescher 1990; Boecking and Drescher 1991; Büchler et al. 1992). To combat this, the parasite is required to maintain a high rate of reproduction within the time and resource limitations of the brood cell. Structuring of the space within the cell helps in this by reducing the time spent by nymphs in searching for a feeding site, and by maximizing energy conservation through minimal displacement of all instars thanks to arrestment on the FA. However, with an increase in the number of families within a given brood cell, *Varroa* runs the risk of overexploiting the host; a dead host or one too weak to emerge imprisons the parasite (Rath 1992). The limited number of drone cells in hives of the original Asian host causes a high rate of infestation, and suggests that multiinfestation is frequent (Tewarson et al. 1992). Here *Varroa*'s group habit and resulting competition at the feeding site may contribute to limiting offspring development. This may explain the reduction in the number of adult progeny per female in multiinfested cells (Fuchs and Langenbach 1989; Moosbeckhofer et al. 1988). The trade-off for *Varroa* in multiinfested cells is the higher probability of finding a male, and consequently gene mixing between surviving progeny (Donzé et al., in prep.). Furthermore, even unmated brood-invading females may lay haploid eggs and thus transfer some of their genes via their sons in multiinfested brood cells.

Although reproduction in *Varroa* is tuned to drone cell development, this does not prevent the parasite from exploiting smaller worker cells either in *A. cerana* or *A. mellifera*. In the case of European honeybee, this exploitation of workers cells is eventually maladaptive when large numbers of workers emerge deformed (De Jong et al. 1982; Koch and Ritter 1991), prone to infection (Glinski and Jarosz 1992) and too weak to perform essential tasks within the hive (Schneider and Drescher 1988), so bee colonies decline.

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