

Time-Activity Budgets and Space Structuring by the Different Life Stages of *Varroa jacobsoni* in Capped Brood of the Honey Bee, *Apis mellifera*

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Varroa jacobsoni reproduces in honey bee brood cells. Here the behavioral activity and use of space by infesting *Varroa* females and progeny were quantified in transparent artificial brood cells. The time-activity budget of both infesting and developing mites converged toward a stable pattern which was established during the bee prepupal stage of the infesting mites and the protonymphal stage of mite progeny. The pattern was such that infesting females and offspring eventually divided their activity between the fecal accumulation on the cell wall, which served as the rendezvous site for newly molted individuals, and the feeding site prepared on the pupa by the foundress. Other parts of the cell wall were used for oviposition and molting, away from the fecal accumulation on which activity of mobile stages was concentrated. Space structuring and the time-activity budget in *Varroa* probably evolved to enhance the number of fertilized females produced within the capped brood, where space and time are limiting factors. These behavioral adaptations parallel those of other mite species which show group behavior within cavities.

KEY WORDS: *Varroa jacobsoni*; Acari; behavior; reproduction; molting, development.

INTRODUCTION

Aggregation within a narrow capsule-like space is a common trait to presocial species of Acari. In such habitats the mites use the available space in an orga-

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nized manner. Several social characteristics are common to these presocial species, such as lack of cannibalism and nest sanitation. These behaviors probably evolved first since they are evidently adaptive in concealed habitats, permitting the development of primitive sociality (Saitò, 1997).

Within the Prostigmata, the mite family Tetranychidae includes parasites of plants which construct sophisticated nest webs where mites of successive generations aggregate and cooperate in nest building, sanitation, and defense. Depending on the species, the web or the plant surface is used for egg laying or defecation. In some species, the mites accumulate their feces at a common site inside or outside the nest, presumably to prevent fouling of resources or to repel predators (Saitò, 1983, 1986). Within Mesostigmata, both *Dichrocheles phalaenodectes* and *Varroa jacobsoni* have a well-developed social organization and compartmentalize the use of the available space. *D. phalaenodectes* parasitizes the tympanic organ of moths, where it uses different cavities for feeding, egg laying, defecation, and molting (Treat, 1975).

V. jacobsoni females parasitize honey bees, reproducing in the capped brood cells. The developing bee in the capped brood is a limited resource, so the parasite must avoid overexploitation. Furthermore, during the limited time span between cell capping and bee emergence, the host passes through four instars, i.e., spinning larva, prepupa, pupa, and adult bee, each differing greatly morphologically (Dade, 1962; Jay, 1962, 1964). *Varroa* is therefore not only confronted with the challenge of reproducing within the limited time span of bee development within the brood cell, but also with quite dramatic changes to the available space due to bee metamorphosis—a factor which increases the risk of mite mortality (Donzé and Guerin, 1994). To combat this the infesting *Varroa* females form an aggregation site with their feces, which they deposit on the cell wall above the bee at the beginning of the prepupal stage. Although the whole length of the cell is available, some 80% of these fecal accumulations (FA) are placed in the posterior part of the cell near the bee's anus. The first egg is laid in the anterior part of the cell at the apex in almost all cells close to the operculum. This anteriorly placed egg runs a lower risk of being jostled by the emerging posterior-pointing pupal appendages during pupation. Following pupation, the infesting females prepare a single feeding site on the abdomen of the bee pupa close to the FA and, in 60% of cells, succeed in moving one or both of the pupal leg III in order to enlarge the space around the FA (Donzé and Guerin, 1994). Since the offspring are unable to feed on their own, they all use the prepared feeding site and aggregate on the FA after each feeding bout. This limitation to feeding at just one site helps to avoid host mortality. In *Varroa*, and probably also *Dichrocheles*, the mites not only defecate on the FA but also use it as a mating site (Donzé *et al.*, 1996; Treat, 1975). The FA can thus be considered as a rendezvous site in these parasitic species.

The well-organized behavior of *Varroa* within the brood cell suggests some

adaptive advantages despite the cost to the founding adults. This might result in conservation of energy by offspring which must reach adulthood within a limited time span. Up to now, little has been established about the actual behavior of mite species showing group behavior within the nest confines, and nothing about the progression of the behavioral patterning of maturing offspring which must one day play the foundress role. Here an account of the behavior of all developmental instars of *Varroa* within the brood cell is presented. We have constructed time-activity budgets for infesting females and developing offspring. The aim of the study is to show how the infesting female adapts her behavioral activity to the developing bee within the brood cell during her reproductive phase, and to demonstrate how the behavior of the offspring is modulated by the structures prepared by the mother.

MATERIALS AND METHODS

Behavioral Observations

Artificial polystyrol cells (internal dimensions: 5.1-mm ϕ \times 14 mm long for worker and 6.7-mm ϕ \times 16 mm long for drones) containing naturally reared brood and infested by *Varroa* within *A. mellifera* colonies were used for continuous observations. After capping, the cells were transferred to a laboratory incubator maintained at 34°C and 60% RH. Humidified air (2 L min⁻¹) entered the bottom of the incubator and two small fans placed at the ceiling ensured on air mix. Direct observations of the artificial cells were made with an operation microscope mounted with a videocamera connected to a time-lapse VHS recorder. All manipulations of cells and optical settings were done through two airtight hand ports in the door of the incubator. The eyepieces of the operation microscope also passed through this door (Donzé and Guerin, 1994; Donzé, 1995). Considering the importance of geotaxis in *Varroa*, the cells were kept in the natural position (i.e., the bee lays on its dorsum) and turned only occasionally for a particular observation.

The observations reported here were made with the help of a computer program which analyzes behavioral events as a function of time [Observer (Noldus, 1991)]. The focal sampling method was employed, and all behaviors described in the repertoire (Table I) were recorded continuously during the observation time. During cocoon spinning by the bee larva and at the prepupal stage, *Varroa* behaviors were directly observed and recorded simultaneously, whereas during the pupal stage all observations were made a posteriori from around-the-clock recordings (3 frames s⁻¹). The position of the mite in the cell, the surface on which it was situated, i.e., the cell wall or the bee cuticle, and its behavior were recorded simultaneously. We report here only on cells where reproduction and development proceeded normally, i.e., with a male offspring

Table I. Ethogram of the Infesting *Varroa* Female.

Inactive	<i>Varroa</i> is immobile and performs no detectable behavioral activity.
Behaviors when immobile	
Cleaning of pedipalps and leg pair I	One of the first legs is held between the pedipalps, which make up-down scissors movements. The pedipalps are rubbed against the first leg only during the downward movement.
Cleaning of leg pairs II to IV	Legs II to IV on one side are rubbed against each other, pairwise.
Feeding	The head region is inclined and pushed against the cuticle of the bee. The chelicerae are introduced in the host cuticle while the pedipalps remain outside on each side of the feeding lesion. Contractions of the intestine are sometimes visible through the body wall.
Displacement behaviors ^a	
Walking, searching	<i>Varroa</i> walks while simultaneously "sampling" the substrate with its pedipalps and first legs.
Repetitive sampling	A succession of short back-forward movements of the whole body without displacement. This results from alternate extension and flexion of legs. Simultaneously, <i>Varroa</i> touches the substrate rhythmically with its pedipalps and leg pair I.
Pivoting	<i>Varroa</i> pivots on itself.
Leg pushing	<i>Varroa</i> abuts against the legs of the newly molted pupa and pushes so fervently that its ambulacra slide on the cell wall or the bee cuticle.
Other behaviors	
Defecating	After relocating the fecal accumulation, <i>Varroa</i> abruptly stops, waggles its anus dorsoventrally, deposits the feces, and stops some 0.5 mm away.
Leg pair I high	<i>Varroa</i> raises its stretched leg pair I; the whole body also has an upward slant from the substrate.
Egg laying	Behavior leading to and including oviposition (Donzé, 1991; Donzé and Guerin, 1994)
Mating	Females and males proceed to the sequence of mating behavior (Donzé, 1995).

^aIn the text the term "displacement" includes the sum of all four behaviors listed in this category.

first and then four females laid successively by the infesting female (Ifantidis, 1983; Rehm and Ritter, 1989, Donzé and Guerin, 1994; Martin, 1995). All observations made during the pupal stage were on cells where the *Varroa* family was located between the pupa's leg pair III and the feeding site was on the fifth segment, i.e., the segment after the propodeum (Fig. 1). Observations began 20 min after the cell was placed under the microscope to avoid recording events possibly related to manipulation of the cell by the experimenter.

Observation periods were chosen after a preliminary study of the entire reproduction cycle and were distributed nonrandomly in time to get the best

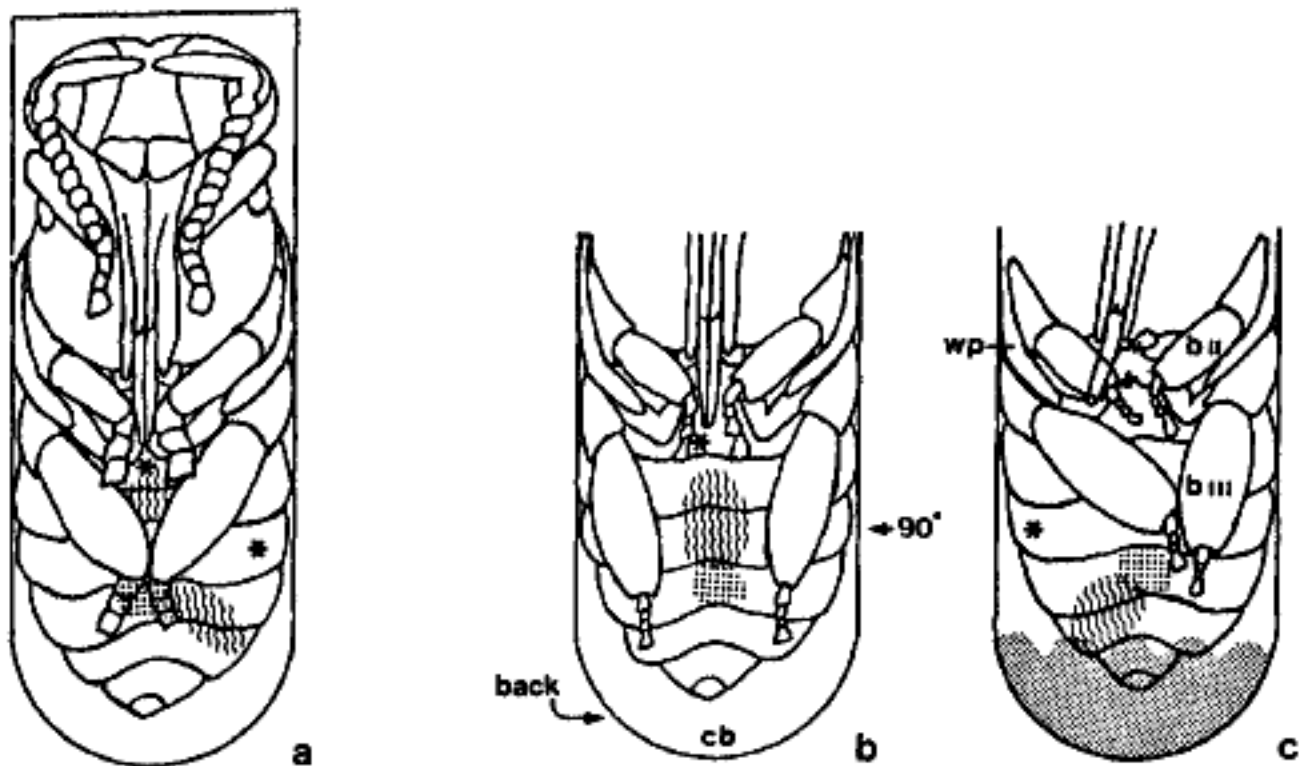


Fig. 1. Defined positions on the cell wall and on the bee during the pupal stage. The small shaded zone indicates the location of the fecal accumulation (FA) on the cell wall formed at the prepupal stage and the adjacent wavy zone represents its extension by the *Varroa* family during the pupal stage. The big shaded zone at the cell base in c represents the bee excrement deposited by the cocoon-spinning larva. Asterisks indicate preferred feeding sites on the pupal abdomen according to the location of the *Varroa* family. As shown by the position of the FA during the pupa (wavy zone) the *Varroa* family is normally situated between or outside leg pair III, which may be in any of the three positions shown in this figure following leg-pushing or not by *Varroa*: (a) leg pair III unmaneuvered by *Varroa* is united over the center of the abdomen (47 and 35% of drone and worker pupae, respectively; $n = 162$ drone and 259 worker brood cells); (b) one or both legs III separated and moved to the sides of the pupal abdomen (27 and 42%, respectively, of drone and worker cells); and (c) one leg III is pushed forward so that more space is available on one side of the pupal abdomen (27 and 24%, respectively, of worker and drone cells). The bee pupa partitions the cell space into an anterior (toward the operculum) and a posterior part at the level of leg pair II. Only protonymphs can pass from one part to the other between tarsi II. cb, hemispheric cell base; bII and bIII, basitarsi of legs II and III; 90° , 90° to the side of the cell; wp, wing pads; back, back of the pupal abdomen or cell wall adjacent to dorsum of the bee abdomen.

description of the changes occurring during the reproduction or development process. For this, we made a series of observations shortly after each morphological change of the bee; less frequent observations were made during periods of no changes to the host. The behavior of *Varroa* described during the latter observations is valid for longer time spans than the observations made following the host's morphological changes. For example, because *Varroa*'s behavior changes remarkably in the 10 h following bee pupation, three observation periods were chosen, whereas during the next 170 h of the pupa stage, when *Varroa*'s behavior evolves slowly, only three observation periods were employed.

Observation on the Infesting Female

Observations on the invading *Varroa* were ordered in hours postcapping (hpc; Fig. 2) as follows.

Spinning Larva. The first observation period began just after *Varroa* came out of the larval food (0–1 hpc), and the three other observations were made during cocoon spinning by the bee larva at about 6, 12, and 24 hpc.

Prepupa. At the beginning of the bee prepupal stage, i.e., from 36 to 48 hpc, four observations were made. During this time the *Varroa* mother copes with the new type of space available in the cell and she forms the fecal accumulation (FA). We also observed the infesting female at the time of first oviposition (70 hpc) and at the end of the prepupal stage (90 hpc). All observations made during cocoon spinning by the bee larva and at the prepupal stage began randomly, i.e., without consideration for the mite's behavioral rhythm, and lasted 60 min.

Pupa. Considering the major changes caused to the space available to *Varroa* by bee pupation, three periods of observation were made after this event. The first began as soon as the pupal appendages were completely extended (96 hpc in Figs. 2 and 6). The second (100-hpc) and the third (103-hpc) observations of the female mite on the young pupa began at the end of her first and second feeding bouts on the pupa, respectively. These three observations lasted about 60 min each and excluded the feeding period. The female was next observed at 160 hpc, i.e., during the period of oogenesis, then shortly after laying of the last egg (200 hpc), and finally, at 260 hpc. Because of the reduced activity of the infesting *Varroa* female during the bee pupal stage, the last three observations lasted 4 h each.

Observations on Offspring

Varroa mites develop through the following instars: egg, mobile protonymph, pharate protonymph, mobile deutonymph, pharate deutonymph, and adulthood. The term mobile nymphs indicates the stages of development during which nymphs feed and grow. Hours postmobilization (hpm) refers to the time after mites have left the hatching or molting site, and immobilization to the time when they stopped on the molting site. Mobile female proto- and deutonymphs take 23 and 27 h, respectively, from onset of hatch or molt until immobilization at the molting site, and pharate nymphal stages last 17 and 48 h, respectively (Donzé and Guerin, 1994). The observations on the mobile female proto- and deutonymphs were made as follows: (a) before the first feed (BF in Figs. 3 and 6), from mobilization until onset of feeding; (b) for 3 h from the end of the first feed (AF); (c) for 4 h some 10 h after mobilization (10), i.e., the typical stabilized behavior of the instar; and (d) during the search for the molting site (BM), starting from the end of the last feed until immobilization at the molting

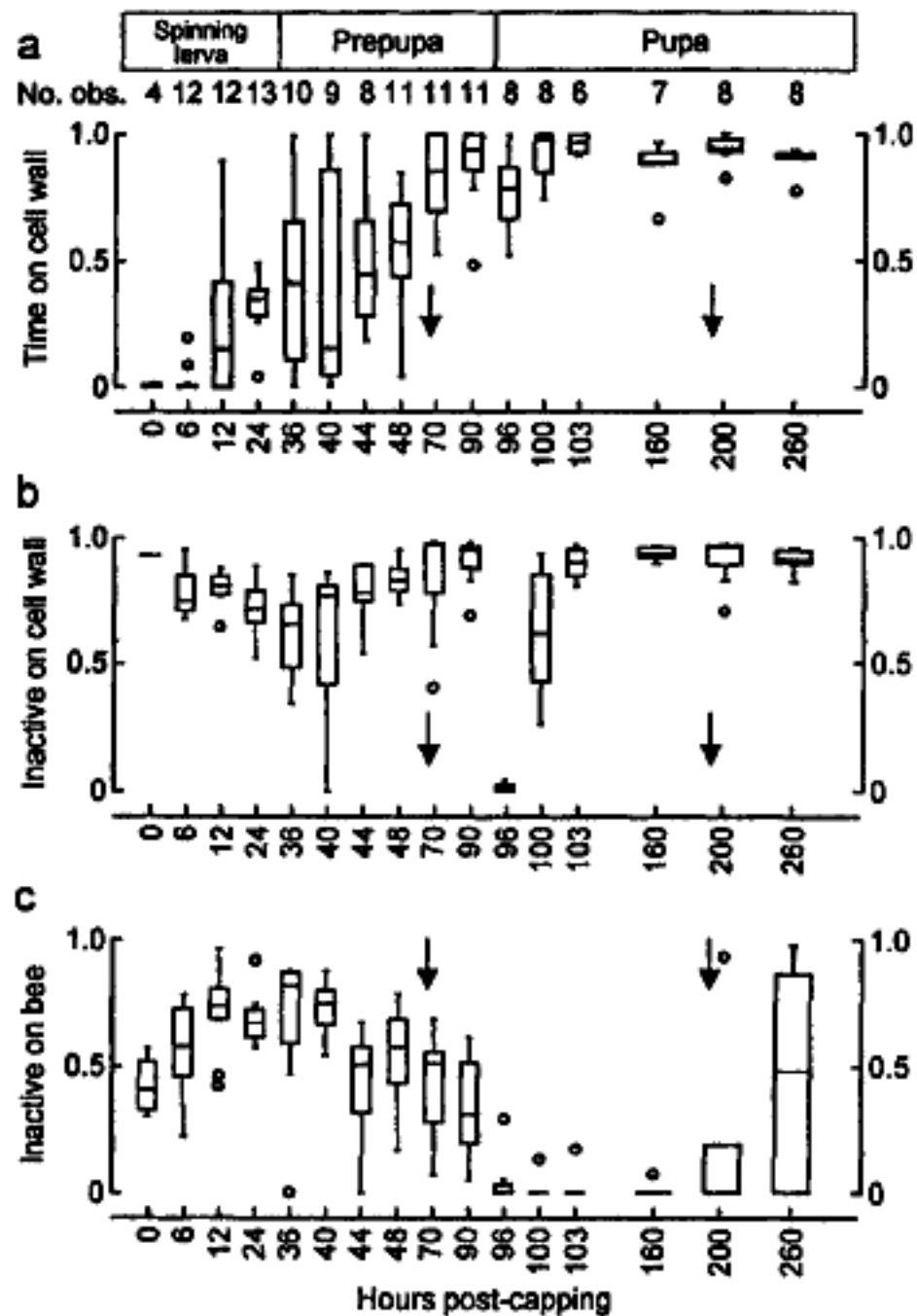


Fig. 2. Behavioral activity of the infesting *Varroa* in the capped brood of the honey bee: (a) time spent on the cell wall as a proportion of the duration of the observation period; (b) time observed inactive as a proportion of the duration on the cell wall; (c) proportion of time observed inactive on the bee. The observations are grouped in periods on the abscissa as a function of hours post-capping (hpc). The number of observations per period is given above the graph. The bee's development is that of a worker, i.e., the prepupal stage begins at approximately 33 hpc and the pupal stage at 93 hpc. *Varroa* lays the first egg, on average, at 69 hpc and the last one at 190 hpc (arrows). The line within the box marks the median, the lower and upper boundaries of a box the 25th and 75th percentiles, and the bars below and above a box the minimum and maximum values except outliers (○).

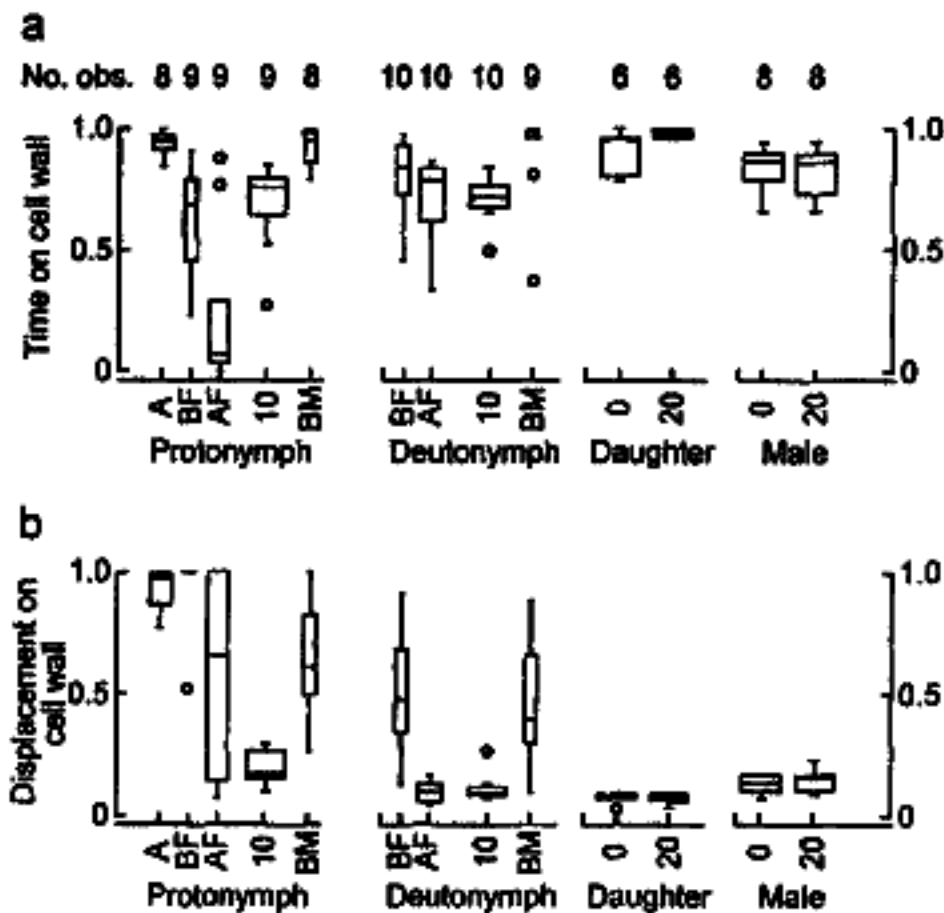


Fig. 3. (a) Time spent on the cell wall by all offspring instars as a proportion of the duration of the observation period. (b) Time spent by the offspring in displacement (Table I) as a proportion of the time observed for the period on the cell wall. The observations are grouped in periods on the abscissa as a function of events or hours postmobilization for each instar: A, protonymphs situated anterior to tarsi II of the pupa; BF, from mobilization until onset of the first feed by protonymphs and deutonymphs; AF, just after the first feeding bout; 0, 10, and 20, hours after mobilization of the nymphs and adults; BM, from the end of the last feeding bout until immobilization at the molting site. The number of observations per period is given above the graph and the width of each box indicates the relative duration of the observation (see text; maximum is 4 h). For details on data distribution see the legend to Fig. 2.

site. In addition, male and female protonymphs which hatched anterior to leg pair II (A) were observed for 4 h before they passed between leg pair II to join the family at the posterior part of the cell.

We observed the young adult mites twice for 4 h: the first observation began as soon as the male or the female left the molting site (0 in Figs. 3 and 6). The second observation of the young females began 20 h after mobilization (20) and that of adult males began when the first daughter arrived on the fecal accumulation, about 20 hpm (20).

Time-Activity Analysis

For each observation, the total duration and the frequency of each behavior displayed on either the bee or the cell wall were calculated. The time during which the mite exhibited a particular behavior was divided by the time during which the mite was situated on the particular surface, i.e., bee or cell wall, to obtain a quotient for the behavior on that surface, which varied from 0 to 1 (or from 0 to 100%). For each observation period, the proportions obtained for each individual were plotted with Tukey box plots (Tukey, 1977; Wilkinson, 1990). This graphical representation of the behavior of all individuals was chosen because it gives the most accurate picture of trends in the behavioral activity of the mites.

Principal-Component Analysis

Each observation period for the infesting *Varroa* and the different offspring life stages can be characterized by the proportion of time spent at each behavior on the cell wall or on the bee. To avoid fragmentation of the behavior, principal-component analysis (Frey and Pimental, 1978) was used. This multivariate analysis permitted us to represent the whole process of behavior modulation during the course of the reproduction cycle of foundresses and during offspring development. The analysis was performed simultaneously for the infesting females and the offspring, but results are plotted separately for clarity.

RESULTS

The behavioral pattern of all infesting females and offspring on the bee differed from their behaviors on the cell wall, therefore, the mites' activity on the two surfaces was analyzed separately. The term arrestment is used here to describe stays by the mites on the FA during which adult mites undertook regular displacements across feces and stopped at the opposite border. This behavior was not observed in nymphs which were inactive for longer periods when on the FA. However, due to aggregation, all mites were regularly pushed by one another and moved off the feces but soon returned to it. A short description of each behavioral category displayed by the infesting females is provided in Table I. We use the term displacement for the sum of all four displacement behaviors unless specified.

Time Spent on the Cell Wall Versus on the Bee

Immediately after her departure from the larval food, the infesting mite adhered to the bee larva and was almost never observed on the cell wall. With time, however, the proportion of time the mother spent on the cell wall increased

progressively and reached a median value of 0.94 at the end of the prepupal stage (90 hpc; Fig. 2a). The variance between individuals was maximal at the beginning of the prepupal stage (36–40 hpc). The proportion of time spent on the cell wall remained high during the whole pupal stage.

All developmental stages and young adults showed a high preference for the cell wall with the exception of the young protonymphs after the first feeding bout (Fig. 3a). This is due to the tendency by all stages to rejoin the cell wall following each feeding bout and to arrest on the FA, but the protonymphs acquired this tendency progressively (see below). Protonymphs situated in the anterior part of the cell were almost always observed on the cell wall (median = 0.95). Soon after the last feed, the proto- and deutonymphs searched for the molting site, which was always situated on the cell wall.

Behaviors of Infesting Females

On the cell wall, the mother was mostly inactive during most of the observation periods (Fig. 2b), whereas on the bee she showed a more varied behavioral activity (Fig. 2c). The first persistent behavioral change of the infesting *Varroa* commenced at about 36–40 hpc with the formation of the FA at the beginning of the prepupal stage and was reinforced thereafter. On the bee, her activity was increasingly focused on searching for the feeding site (increase from 0.05 at 0 hpc to 0.51 at 160 hpc) and subsequent feeding (from 0.007 at 40 hpc to 0.49 at 160 hpc) while the time spent inactive there decreased (Fig. 2c). On the contrary, the mite used the cell wall increasingly when inactive (Fig. 2b), cleaning itself and defecating. This molding of the female behavior was well illustrated by the length of time which separated the end of a feed and the arrival on the FA: the duration of the actual trip shortened to just 20–30 s at the pupal stage as compared to 250 s at 50 hpc (Fig. 4). As a consequence, the proportion of time the mother spent on the FA increased from 0.1 at 48 hpc to 0.9 at 90 hpc and this lasted until at least 160 hpc (Table II). At the end of the prepupal stage, the *Varroa* mother demonstrated the following behavioral rhythm: when hungry she descends to the bee where she searches for the feeding site and feeds. After feeding, she returns to the FA where she arrests. This routine was strictly maintained until the end of oogenesis, except for the short period following bee pupation.

The second durable modification of the mother's behavior occurred following deposition of the last egg. After the last oviposition (200 + 260 hpc), the mother either stayed inactive on the bee (Fig. 2c) or on the cell wall, but removed from the FA (Table II). This was facilitated by the shrinking of the pupa so that more space was available between basitarsi III and the cell wall. She did return regularly to the FA to defecate and to arrest, but after an encounter there with another individual the mother moved away from the FA to the side of the cell.

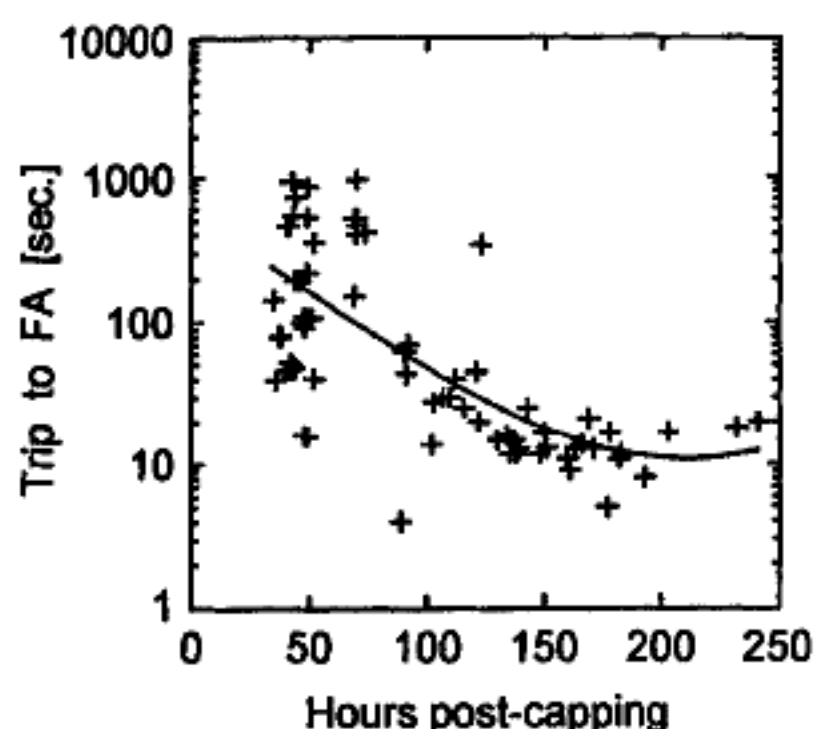


Fig. 4. Trip duration of the infesting *Varroa* from the feeding site until arrival on the fecal accumulation plotted against hours post-capping. The trend line was calculated with the distance-weighted least-squares method (Wilkinson, 1990). The infesting female regularly stayed inactive after feeding at different sites within the brood cell at the beginning of the prepupal stage (36–48 h postcapping) but later returned directly to the FA.

Table II. Time Spent by the Infesting *Varroa* Female on Three Zones of the Cell Wall^a

Hours postcapping	n	On FA		Cell wall over Basitarsi III		90° on the sides of cell wall	
		Median	Min.-max.	Median	Min.-max.	Median	Min.-max.
48	11	0.1	0–0.8				
70	11	0.7	0.3–1				
90	11	0.9	0–1				
160	7	0.9	0.8–1	0	0–0.1	0	0–0
200	8	0.5	0–1	0.1	0–0.6	0	0–0.4
260	6	0.2	0.1–0.3	0.4	0.1–0.8	0.2	0–0.8

^aThe proportion of time for each position is calculated as a function of the total time spent on the cell wall: median and minimum–maximum values; n = number of individuals observed. The three sites are *on FA*, which represents the fecal accumulation and its environs; *Basitarsi III*, the zones of the cell wall situated above the two basitarsi III of the pupa; and *90° on the sides*, the lateral zones on the cell wall outside of the basitarsi III. For further explanation see also the legend to Fig. 1. Observations were made at 48, 70, and 90 h postcapping during the bee prepupal stage and at 160, 200, and 260 h postcapping during the pupal stage.

The reaction of *Varroa* to bee pupation merits some details. Due to the amount of time spent by *Varroa* in pushing leg III of the pupa laterally (Table I; "leg-pushing"), the amount of time spent in displacement on the cell wall increased from 0.015 before bee pupation (90 hpc) to 0.98 at the end of pupation (96 hpc; Fig. 2b). Prior to the first and the second feeding bouts on the young pupa, the leg-pushing behavior represented a mean of $70 \pm 14\%$ ($n = 8$) of the total displacement on both substrates and led in some cells to shifting of one or both legs III (Fig. 1). The frequency and the duration of the leg-pushing bouts varied as follows: before the first feed it occurred 37 ± 18 times h^{-1} for 71 ± 38 s bout $^{-1}$, while after the first feed it occurred only 5 ± 5 times h^{-1} for 179 ± 118 s bout $^{-1}$ ($n = 8$ individuals). This behavior eventually disappeared, independent of whether the position of the bee's appendages was modified.

Behaviors of Protonymphs

After hatching, the protonymphs stayed at the oviposition site for several hours. Once they left it, all protonymphs located either in the posterior or in the anterior part of the cell were highly active on either the cell wall or the bee and in all cases the displacement behaviors represented $\geq 97\%$ of time (Fig. 3b). Of nine protonymphs observed in the posterior part of the cell, only two stopped once; all the others moved continuously until the onset of their first feed. At first, protonymphs do not arrest on the FA. Despite the long duration of the search for the feeding site (54 ± 43 min; $n = 9$), protonymphs rarely strayed into zones far from the FA and the feeding site. When they approached the feeding site they did not always succeed in locating it at first but showed intense local search activity. We could not detect any methodical searching behavior on the part of the protonymphs situated in the anterior part of the cell. They were very active until they joined the family or, if the protonymph did not succeed in locating the passage between tarsi II, until death occurred approximately 20 h after mobilization. Once such protonymphs had passed posteriorly between tarsi II, the level of search activity remained high until they had fed for the first time.

After the first feed, protonymphs stayed inactive on the pupa near the feeding site (18 of 19 individuals observed). Here, the protonymph prevented other mites from accessing the feeding site, and in 13 of 29 cases they were consequently disturbed. Protonymphs stayed inactive near the feeding site for a significantly shorter duration when disturbed (42 ± 26 min; $n = 12$) than when undisturbed (78 ± 35 min; $n = 16$; $P < 0.01$, Mann-Whitney U test). After being disturbed once or several times by another mite, all protonymphs moved, and 8 of 13 climbed onto the cell wall, where they subsequently arrested on the FA. The number of protonymphs remaining on the bee decreased to 10 of 19

after the second feeding bout and to 1 of 19 after the third and fourth feeding bouts. Thereafter, all protonymphs returned immediately to the FA after each feeding bout. Protonymphs which stayed on the bee after the first feed did not find the feeding site more quickly than those who visited there from the FA ($P > 0.5$, Mann-Whitney U test; $n = 5$ and 6 ; with medians of 870 and 995 s, respectively), as all were observed searching on both substrates before the second feed.

Ten hours after mobilization the protonymphs exhibited a different activity according to the substrates on which they were situated. When on the bee, they displaced for 30% and fed for 68% of the observation time (medians). Thus, as observed for the mother, the protonymphs eventually only went onto the bee for feeding. In contrast, they were inactive for some 83% of the time on the cell wall.

Behaviors of Deutonymphs

Deutonymphs showed less variation in their behavior than protonymphs. Once they left the molting site, 8 of 10 deutonymphs immediately rejoined the FA, where they arrested for 18 ± 17 min ($n = 10$) before moving off in search of the feeding site. Thus, in the period prior to their first feed, the deutonymphs walked 100% of the time while on the bee, whereas they walked only during 48% of the time spent on the cell wall (median values). The set of behaviors acquired during the protonymph stage, i.e., to return to the FA after feeding, was conserved. After the first feed, 8 to 10 returned to FA, after the second all 10, and after the third 9 of 10 deutonymphs returned immediately to the FA where their displacement was low (median = 0.1; $n = 10$; Fig. 3b). Deutonymphs stayed 79–87% of time on the FA for most of the life-stage until the search for a molting site began.

Behaviors of Adult Offspring

Just after mobilizing, young adults rejoined the FA where they demonstrated arrestment. For the first 4 h they spent 75% of the time on or near the FA ($n = 8$ males and 6 females). Here the behavior of the adult offspring was characterized by regular crossing of the FA. Males were more often active than females, but both sexes stayed almost always on or near the FA. The first feeding bout occurred in males 4.3 ± 2.7 h after mobilization and males stayed inactive on the bee more often than the adult daughters.

Other Types of Behaviors

Just after liberation from the larval food, the infesting mite spent 30% of the time cleaning herself, and 6 h later this proportion dropped to 10% (medi-

ans). The cleaning behaviors were difficult to observe in proto- and deutonymphs, but young females regularly showed pedipalp and leg cleaning behaviors, mostly when on the FA. Prior to copulation, the male often cleaned his chelicerae and pedipalps.

Both infesting mites and adult daughters were observed to pivot and wagged the anus dorsoventrally before defecation on the FA. Although all other instars were also observed defecating on the FA, neither nymphs nor males pivoted or wagged.

Molting Site Location

The nymphs returned to the FA after the last feed and then began a search activity which took place almost exclusively on the cell wall (medians = 0.96 and 0.97 for proto- and deutonymphs, respectively). Some mites traveled along the FA border (Fig. 5) before leaving for the molting site, whereas others criss-

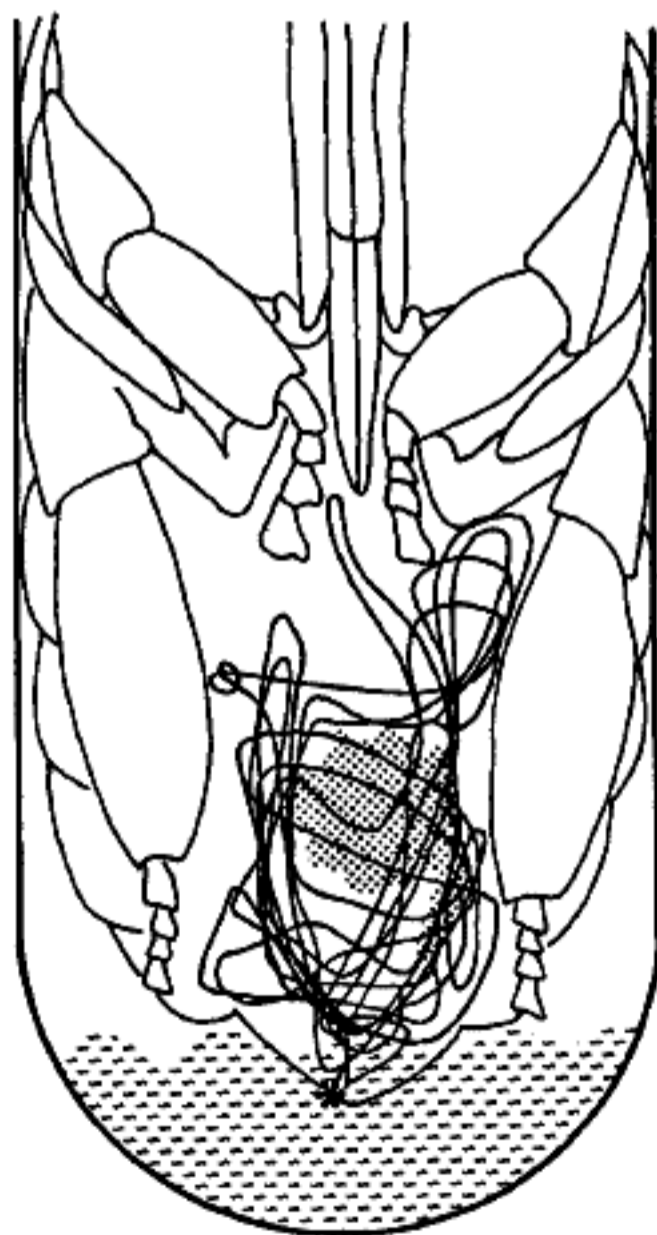


Fig. 5. Track made by a protonymph on the cell wall prior to immobilization at the molting site (asterisk) at the border of the bee's excrement. Track started on the fecal accumulation (FA) and ended at the border of the bee excrement. The shaded zone represents the mite FA on the cell wall; the wavy zone at the cell base represents the bee excrement deposited by the cocoon-spinning larva. Stops made by the protonymph are not indicated.

crossed regularly between the FA and the wing pads or between the FA and the border with the bee's excrement. This search activity occurred in bouts with stops (6.9 ± 3 and 9.6 ± 3.6 bouts h^{-1} for proto- and deutonymphs, respectively, compared to 2.3 ± 0.8 and 4 ± 1 walking bouts h^{-1} during the previous observation period; mean \pm SD). The net result was more frequent visits by the nymphs to different parts of the cell (e.g., before molting, proto- and deutonymphs reencountered the FA 13.5 ± 8 and 15.8 ± 10 times h^{-1} , respectively, compared to 1.7 ± 0.7 and 2.2 ± 1 reencounters h^{-1} in the previous observation period, and were observed 6.5 ± 4.1 and 9.1 ± 5.9 times h^{-1} , respectively, on the cell wall above the wing pads, compared to 0.2 ± 0.1 and 0.2 ± 0.4 times h^{-1} in the previous observation period). Eventually the nymphs left the FA region for a molting site, which always was on the cell wall and distant from FA. During the latter part of this trip the nymph moved almost imperceptibly and as far as possible in the direction of the molting site. Table III summarizes the preferred molting sites according to the position of the *Varroa* family. Deutonymphs molted at a greater distance from the FA than the protonymphs and a higher proportion reached the cell wall region adjacent to the back of the abdomen of the pupa.

Principal-Component Analysis

The data for all behavior types (considered separately for the cell wall and the bee) plus the total time spent on the cell wall, on the bee, and on the FA

Table III. Positions of Eggs and Pharate Proto- and Deutonymphs on the Cell Wall^a

Instar	n	Back of cell (%)	Cell base (%)	Border with bee's excrement (%)	Sides of cell (%)	Along wing pads (%)
<i>Varroa</i> family situated outside of leg pair III						
Egg	27	11.1	18.5	44.5	14.8	0
Pharate protonymph	22	36.4	27.3	0	18.2	4.5
Pharate male deutonymph	39	85.7	9.5	0	0	0
Pharate female deutonymph	21	71.8	5.1	0	10.3	10.3
<i>Varroa</i> family situated between leg pair III						
Egg	53	1.9	9.4	47.2	24.5	15.1
Pharate protonymph	64	15.6	6.2	12.5	17.2	32.8
Pharate male deutonymph	69	62.5	3	3	6.2	12.5
Pharate female deutonymph	32	42	5.8	4.3	14.5	24.6

^aThe cells are grouped according to the position of the *Varroa* family during the pupal stage (see Fig. 1). Values indicate the percentage of individuals for each instar. Additional sites and sites difficult to classify are omitted; n = number of individuals observed.

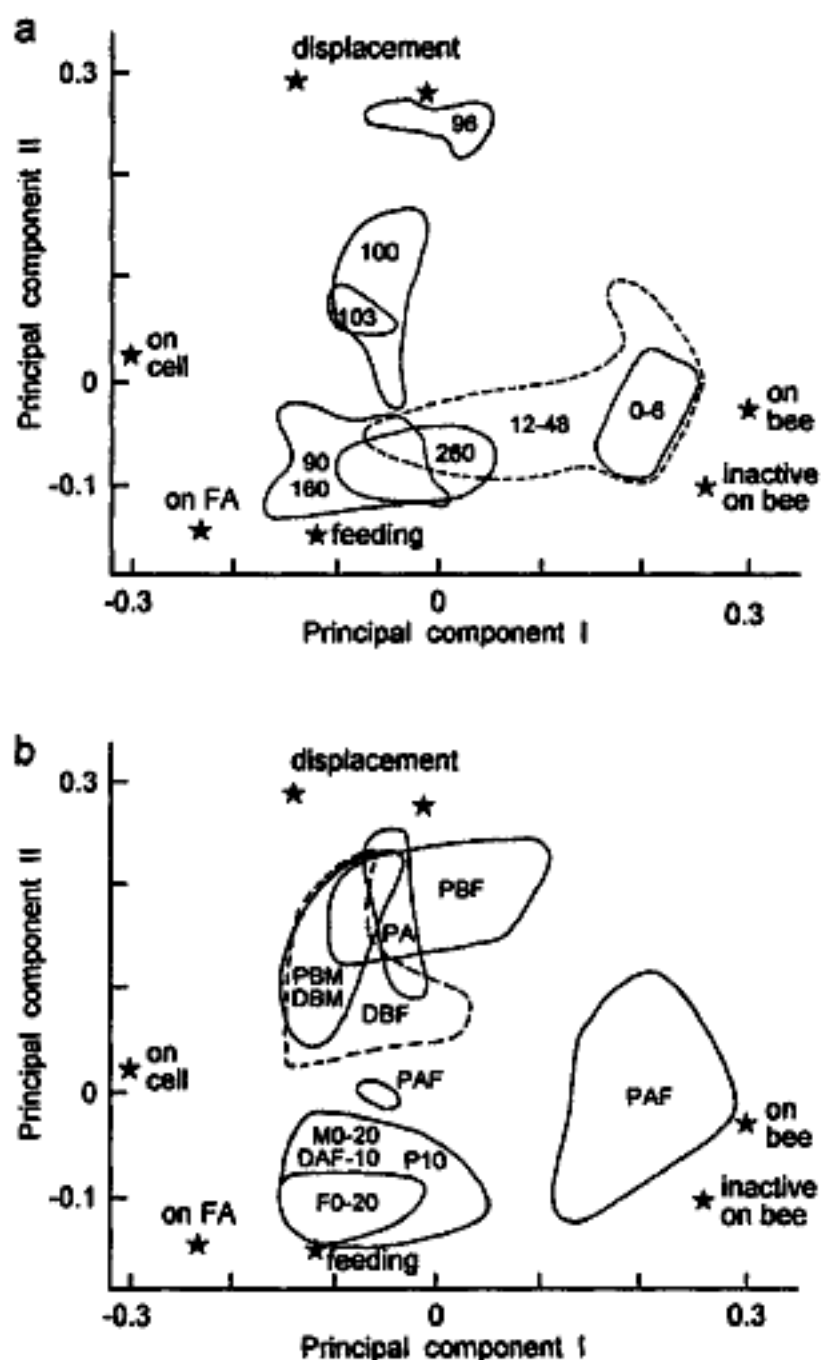


Fig. 6. First two principal components of the time-activity budgets. (a) Infesting *Varroa* from cell capping (0 h postcapping; hpc) until the advanced bee pupal stage (260 hpc). For clarity, some observation periods are grouped into one cluster and two are omitted (at 70 and 200 hpc). (b) Developmental stages of *Varroa* offspring. Observations periods: BF, from mobilization until onset of first feed of protonymphs (P) and deutonymphs (D); AF, from the end of the first feeding bout; BM, from the end of the last feeding bout until immobilization of the nymph on the molting site; 0, 10, and 20, hours after mobilization of the nymphs, adult male (M), or adult female offspring (F). PA signifies protonymphs situated anteriorly to leg pair II of the bee pupa and stars indicate the positions of substrates and of principal behaviors. Inactivity on the cell wall is situated outside the plot: -0.13×-0.27 .

were analyzed simultaneously for infesting females and offspring. The results indicate that the first principal component accounted for 39% of the total variance and the dominant variables were "on cell wall" (weighted, -0.31) and "on bee" ($+0.31$) (Fig. 6). The second principal component absorbed 31% of the total variance and the dominant factors were "inactive on cell wall" (-0.27), "displacement on bee" ($+0.29$), and "displacement on cell wall" ($+0.28$).

The behavior of the infesting female evolved progressively along the first principal component (from bee to cell wall). More time was again spent on the bee after the last oviposition (>190 hpc). The females were predominantly inactive except for the period following bee pupation (96–103 hpc) when displacement dominated (Fig. 6a).

The behavioral activity of offspring showed two main clusterings: (1) when nymphs initially searched for the feeding or molting sites (A, BF, BM; Fig. 6b) and (2) when they arrested mainly on the FA and fed on the bee (10, adult offspring). The analysis shows that all life-stages used the cell wall preferentially except for the protonymphs after the first feed (PAF).

DISCUSSION

Behaviors of Infesting Females

The behavior of the infesting female just after its liberation from the larval food showed two interesting aspects. First, the mite never tried to enter the narrow space between the bee larva and the cell wall, although this behavior permitted the female mite to invade the brood cell only a few hours earlier. Later, however, the parasite moved perhaps to avoid being crushed by the bee larva when the latter undertook somersaults during cocoon spinning. Since oogenesis begins very early and the female grows heavier (Akimov *et al.*, 1990; Steiner *et al.*, 1994), the oocytes may be particularly sensitive to damage. Second, the females were observed exclusively on the bee larva for the first 6 hpc. This preference for the bee is coherent with the attractivity of the larva for the mite (Le Conte *et al.*, 1989; Rosenkranz, 1990; Rickli *et al.*, 1992, 1994).

Due to the strict oviposition regime in *Varroa*, the time span between the end of cocoon spinning by the bee and the laying of the first egg by *Varroa* is shorter in drone than in worker cells (Donzé and Guerin, 1994). From the end of cocoon spinning the female concentrated her activity in the posterior part of the cell and soon formed the FA. The first egg was subsequently placed away from the FA in the anterior part of the cell. One possible advantage of this is that as the activity of other foundresses was also concentrated on the single FA in multiinfested cells, the risk of disturbance by them to the eggs was reduced. Arrestment on the FA increased with time, and *Varroa* behavior depended on the substrate used: searching for the feeding site on the bee and inactivity or

cleaning itself on the FA. The stability of the behavior induced by the FA is obvious, and despite the abrupt changes induced in *Varroa*'s behavior during bee pupation, the mite was only temporarily distracted from arresting on the FA. On the young pupa the *Varroa* mother's activity was elevated and she moved everywhere in the available space above the abdomen. However, the available space left by the bee pupa was small so the motivation to engage in enlarging the space above the pupal abdomen and permitting access to the preferred feeding site increased with time. As she had not fed for a relatively long time during pupation, she probably lacked the force for long leg-pushing bouts initially. After the first feed on the pupa, leg-pushing bouts of longer duration were recorded. During this time the infesting female has to cope not only with the consequences of bee development but also with her own progeny. In drone cells, the third egg (the second one in the worker brood) is laid on average 10 h after pupation (Donzé and Guerin, 1994; Martin, 1995) and the first protonymph, unable to feed on its own, is already seeking a feeding site. It will not survive longer than 20 h without a meal, so the infesting female must assure availability of structures, i.e., space around the FA and the feeding site.

After the last oviposition act the behavior of the female changed, as she remained inactive at a location removed from the FA. We must relate these events to what happens in some drone cells at the end of the bee's development (~320 hpc). In such cells the mother moves to the anterior part of the cell, where she deposits her feces at random on the cell wall and even sometimes on the bee (Donzé, unpublished data).

Two hypotheses may be proposed to interpret the two long-lasting trends which occurred in the behavior of the infesting female during her reproduction cycle, i.e., concentration of behavior on the FA and feeding site, and the dropping of this pattern after the last oviposition act. The first hypothesis is that the behaviors are regulated endogenously. When oogenesis occurs *Varroa* divides its behavior between the cell wall where it lays eggs and the bee where it feeds. After the last egg is laid, the FA and the cell wall may lose the interest of the mite, which prepares itself for the next phase, i.e., parasitizing adult bees. Cues that trigger oogenesis in *Varroa* and those that end it are unknown. However, it is known that specific behaviors can induce ovarian development in arthropods (Scott and Traniello, 1987) and that feeding status or oogenesis can influence the behavior of insects (see Blaney *et al.*, 1986; Klowden, 1990).

The second hypothesis is that the behavior of the mites is influenced by chemostimuli (exteroceptive influences). We know that *Varroa* reacts to olfactory stimuli, such as aliphatic esters and palmitic acid, and to long-chain hydrocarbons, which acts as contact chemostimuli from larvae (Le Conte, 1989; Rickli *et al.*, 1992, 1994). Some of these olfactory chemostimuli reach a maximum release rate at cell capping and diminish by the spinning larval stage (Trouiller *et al.*, 1992). At the same time, as the bee larva spins its cocoon on the cell

wall, it deposits secretions other than the cocoon webbing (Jay, 1964) and defecates at the cell base at approximately 10–15 hpc. Recent studies have clearly shown that cocoon extracts contain chemostimuli arresting *Varroa* (Donzé *et al.*, unpublished data). Therefore, the decrease in time spent on the bee by *Varroa* during cocoon spinning could reflect a loss of larval attractivity to the advantage of the more favored cell wall. This is subsequently reinforced during the prepupal stage, when *Varroa* concentrates on defecating at one location on the cell wall (Donzé and Guerin, 1994).

Behaviors of Developmental Stages

The behavior of nymphs evolved quickly to a defined pattern characterized by two components, according to the substrate used by the mites. After feeding, the mites returned to the cell wall, where they arrested on the FA. On the bee, however, searching and feeding behaviors represented the main occupations. This fixed pattern was reached after the third or fourth feed by protonymphs and after the first feed by deutonymphs and adults. Once acquired, this behavioral pattern was maintained during the rest of the instar except after the last feed, when the mite searched for the molting site. The behavior at the beginning of each nymphal stage can be considered as a period of transition to the stable phase of nutrition and growth. The results show that arrestment on the FA was inhibited only by the need to feed, as shown by the very high activity of protonymphs prior to their first feed.

The protonymphs did not seek out the cell wall after the first feed. Several reasons for this behavior can be proposed. Following the long search for the feeding site, followed by feeding activity, the mite may have been exhausted. Moreover, because of its increased weight, an engorged protonymph is more likely to fall from the cell wall, where it walks upturned. A functional hypothesis could be that by staying on the pupa, the protonymph could localize the feeding site more quickly for the next feed than those coming from the FA, but our results do not support this. By staying near the feeding site the protonymph prevented other mites from approaching it and, so, entered into conflict with cohabitants. The disturbance by other individuals eventually caused the protonymph to leave the bee for the cell wall, where it encountered the FA. Protonymphs do not accept disturbance on the bee but do so on the FA. This is an indication that the expense associated with interindividual disturbance on the FA is rendered worthwhile due to the overall benefit accruing from group behavior in the capped brood (Donzé and Guerin, 1994; Donzé *et al.*, 1996).

Convergence of the Time-Activity Budgets in All Life Stages

The most important aspect of the behavior of all *Varroa* instars is the structured use of space within the cell. We can compare the behavior of *Varroa*

when returning to the FA after feeding with that of other arthropods returning to their nests *sensu lato* [philopatry (Michener, 1974; Buskirk, 1981; Saitò, 1986)]. The behavior of the mother itself stabilized only after the FA had been formed, and thereafter her behavioral activity was disturbed only temporarily by bee pupation. The relatively short time the protonymphs required to establish their behavioral pattern may have been facilitated by the manner in which the cell compartment had been structured by the infesting female. The principal-component analysis showed that the time-activity budget of the infesting female shifted principally from the bee to the cell wall (principal component I), while that of all developmental stages changed from displacement (search activity) to inactivity on the cell wall (principal component II). But frequent trips between the FA and the feeding site require energy. This is certainly a disadvantage since the unfed developing bee is a limited food resource which the parasite must avoid overexploiting. Learning the way between the FA and the feeding site serves to reduce time and energy costs. Learning occurs during the bee prepupal stage, when the infesting female shortens the time separating the end of feeding and her arrival on the FA. It also occurs in nymphs, which acquire the behavioral routine and increase their ability to localize the feeding site efficiently (reduced from >1000 s to 200 s during the two life stages). Since the deutonymphs and issuing adults soon adopt the behavioral routine acquired during the protonymphal stage, this supports the hypothesis that what is learned is conserved transstadially. This adaptation probably serves the mites to cope better with the competition occurring at the feeding site as they get older (Donzé and Guerin, 1994). Therefore, the time-activity budget of mites may contribute to shorten the development time and to reduce energy consumption. This in turn may serve to enhance the number of fertilized daughters produced before bee emergence. As a consequence, it is plausible to suggest that the oldest daughters in the cell, which have more time to feed and to practice the behavioral routine before bee emergence, will fare better as foundresses in organizing the space of their own newly colonized brood cells.

Space Structuring and Reproductive Success

Varroa and *Dichrocheles* reproduce in the small space provided by the capped brood and the moth tympanic organ, respectively, and both species present similar space structuring. These species feed at specific zones conditioned by access to the host hemolymph and prevent staining potential feeding sites with excrement by concentrating their feces at one spot. The feeding site melanizes on some bees, thus forcing the mites to prepare a new one. Disseminated feces on the pupa could reduce feeding site possibilities and could potentially plug stigmata of the pupa. We have shown here the fundamental contribution of the FA to establishing the behavioral pattern of all instars. But

arrestment behavior on the FA was inhibited on two occasions: first, when nymphs searched for a molting site and, second, when the mother searched for an oviposition site. On these two occasions the mites crossed the FA or traveled along its border before leaving it, and these tracks led either to oviposition or to molting sites removed from the FA. This behavior prevents placement of eggs or immobilization of pharate nymphs at the focus where the activity of the family is concentrated. The high frequency at which deutonymphs molt at the back of the cell suggests its advantage as a protective crevice in which to reduce mortality following disturbance during the molt.

Varroa lays five or six eggs (Ifantidis, 1983; Martin, 1995), of which the first is always the single male (Rehm and Ritter, 1989). While this optimizes the number of female offspring, it could lead to a lack of adult males to fertilize the females either due to developmental mortality of the single male or to nonencounter of the male with females (Fuchs and Langenbach, 1989; Donzé *et al.*, 1996). The latter could happen as the small space available for *Varroa* in the brood cell is divided into two parts by the body and leg pair II of the pupa. But encounter of the sexes is assured by the rendezvousing of all mites on the single FA (Donzé and Guerin, 1994). In addition, the behavioral routine adopted by all offspring causes early and frequent encounters between adults following molt. Rematings augment the number of spermatozoa stocked in the spermatheca, which in turn increases the potential fecundity of emerging females (Donzé *et al.*, 1996). In this context it is interesting to note that the infesting females distances herself from the FA after the last oviposition, thereby reducing the time invested in abortive mating acts between son and mother.

Donzé and Guerin (1994) and Saitò (1997) refer to the mutualistic aspect of the mites' behavior since the common use of nest structures by all individuals permits fertilization of the highest number of offspring (Donzé *et al.*, 1996). Furthermore, a single feeding site means that the risk of host mortality due to rupture is kept to a minimum. Since the mites leave the feeding site once they have fed, a maximum number of individuals have access. In cells infested by two or three females, the reduced number of offspring which eventually mature is largely compensated for by the higher probability that at least one male reaches adulthood within such cells. Consequently, the number of fertilized daughters per infesting female is kept high and inbreeding is reduced (Donzé *et al.*, 1996). The time-activity budget and space structuring recorded here for *Varroa*, which also occurs in other presocial mites, may have evolved from the several advantages which accrue.

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