Varroa Defense Behavior in *A. mellifera carnica*

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Summary

The method of using Infra-red photography was found very useful for conducting long-term behavioristic studies of honey bees against *Varroa*. The photographs show the defensive ability of this species and solved the earlier suspicion of direct damage. In total 30% and 26.6% artificially infested cells were uncapped and removed in two experiments. The correlations between uncapping and removal, uncapping and grooming and removal and grooming behavior were found to be r=0.37, p=0.001; 0.03, p=0.5146 and 0.01, p=0.8179, respectively. Large significant differences between colonies w.r.t. uncapping, removing and grooming and very similar results of three colonies in two experiments indicates a genetic basis for these traits. The correlations between percentages of damaged mites under field conditions and test performance of their individuals with regard to uncapping, removing and grooming were estimated to be 0.63 (p=0.07, n=9), 0.43 (p=0.25, n=9) and 0.72 (p=0.11, n=6), respectively. The outstanding performance of a few individuals in expression of various traits indicates their usefulness in carrying out breeding programs for *Varroa* tolerance.

Introduction

The ectoparasitic mite *Varroa jacobsoni* Oud has posed a serious concern and threat to the *A. mellifera* beekeeping industry. *A. cerana*, its original host, has evolved both physiological and behavioral adaptations to parasitism of *Varroa jacobsoni* (Peng et al., 1987). Damaged mites were observed by various workers on the bottom of infested *A. mellifera* colonies (Ruttner and Hänel, 1992; Büchler, 1993; Fries et al., 1996) indicating an active defense against *Varroa*. Damage was assumed to be the ability of *A. mellifera* to kill mites, but with doubt (Ruttner and Hänel, 1992; Büchler, 1994), since this was also attributed to various other factors including ants and other predators.

Previous workers studied grooming behavior, one of the defense traits, using glass-walled observation hives (Peng et al., 1987; Bozic and Valentincic, 1995; Fries et al., 1996). Comparing some of the *Varroa* resistant traits, percentage of damaged mites falling on the bottom board of different colonies was found to be a good trait and a useful method for beekeepers to sort out *Varroa*-tolerant colonies (Bienefeld, 1996a). Szabo et al., (1996) used transparent plastic containers to test hygienic bees and suggested that the rate of mite expulsion and presence of damaged mites could be used to assess a colony's *Varroa* resistance potential.

We observed the assumed defense mechanisms of *A. mellifera carnica* with the help of infra-red photography for resolving the above stated controversy. This technique was used rather than visible light, being undetectable by bees and not disturbing them dur-

Figures 1, 2, 4a, 4b, 5, 7 and 7a are infra-red photographs showing defensive ability of *Apis mellifera carnica*.

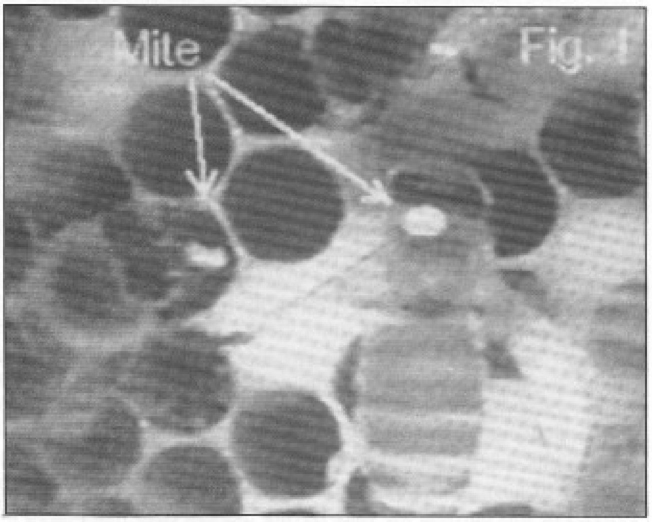

Fig. 1. View of a bee holding and biting a mite with its mandibles.

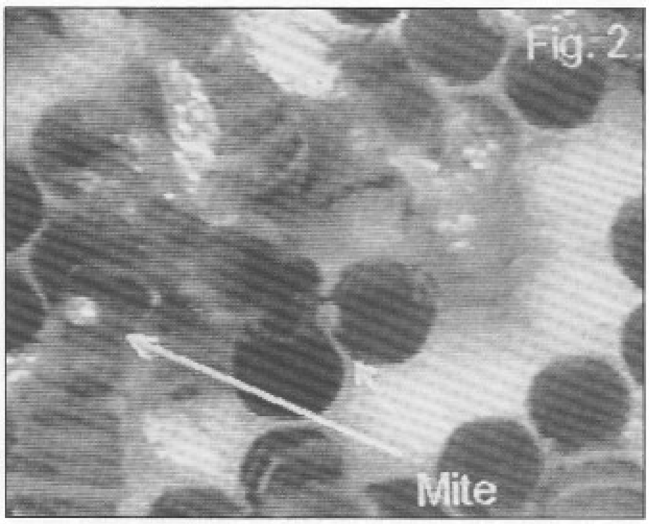

Fig. 2. Bee in the center picking up a free-moving mite on the rim of the cell.

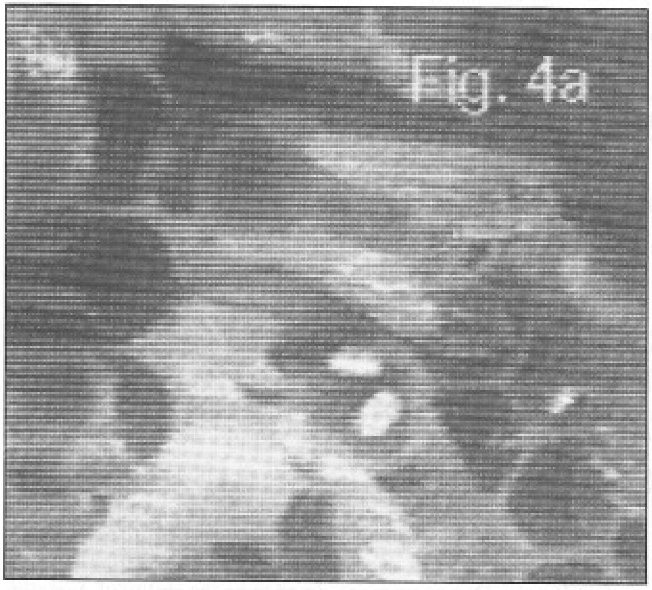

Fig. 4a. Bee infested with two mites.

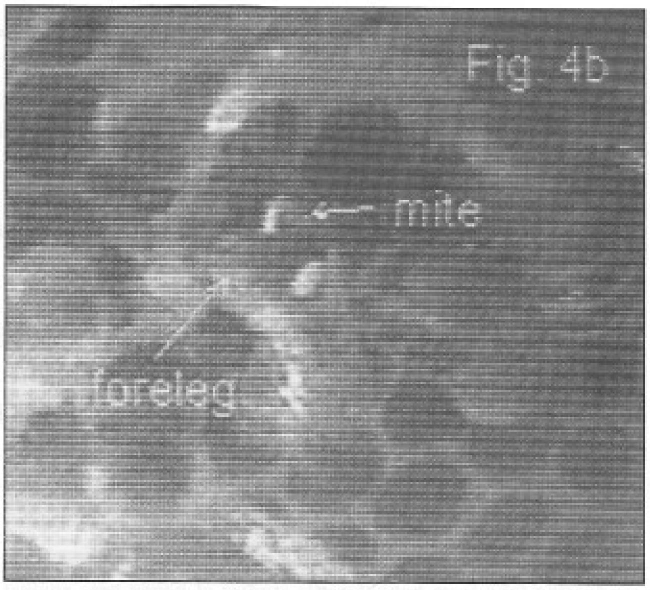

Fig. 4b. Example of mite disturbed by the bee's foreleg (self grooming).

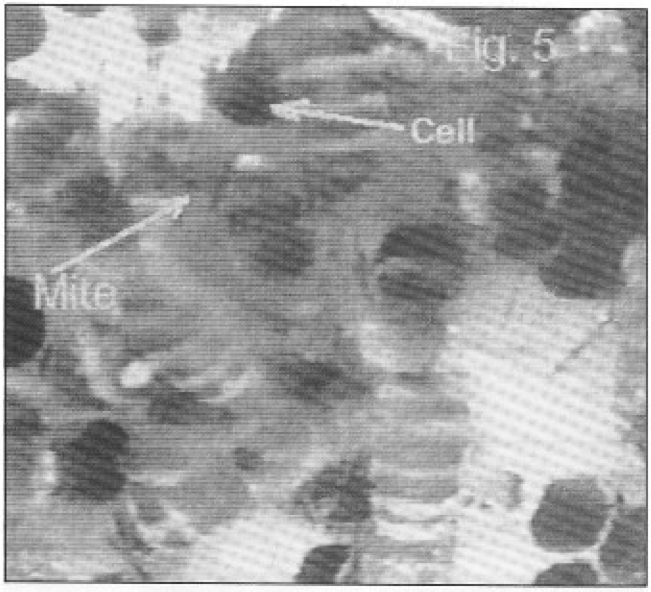

Fig. 5. A mite taken out of the cell and the bee biting it.

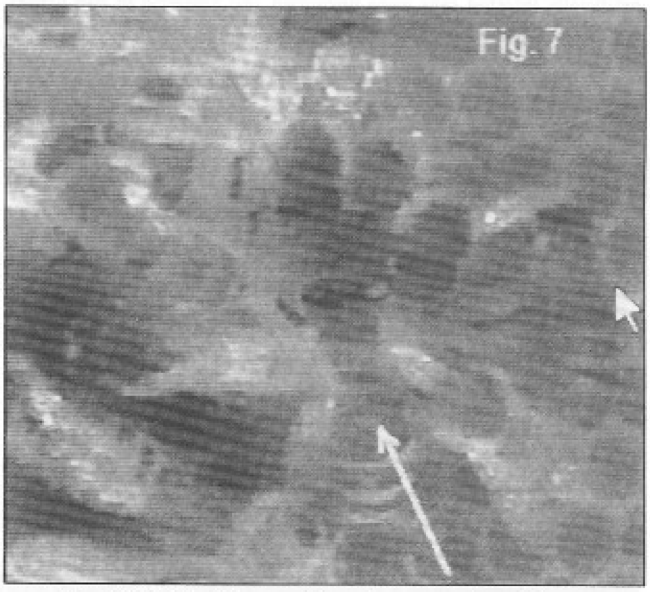

Fig. 7. Infested bee performing grooming dance.

ing long-term observations (Thakur et al., 1996a). These studies were designed to video-record and analyze the defense abilities of this species against *Varroa*, and to determine some genetic basis for occurrence of various *Varroa* defense mechanisms.

Materials and Methods

The defense mechanism was investigated by maintaining bees in a one frame cage with sliding frontal glass and metal gauze behind. The sliding glass was provided with a window for providing food to the bees and artificial *Varroa* infestations. The bees were placed on a brood frame having both sealed and unsealed brood. The cage was placed in a polystyrene nuclei with four bee frames including one adjacent brood frame. This six frame polystyrene mating nuclei, having sufficient bees and a queen, was prepared three days prior to the start of the test. Twenty-four hours before start of the test, the queen was shifted to the cage for facilitating better harmony and stabilization of the system during long-term obsevations. Honey and pollen were provided continuously to the bees at the base of the cage. The infra-red camera was placed on one side of the mating nuclei, facing the glass side of the cage.

The miniature camera (4x4x3 cm) consisted of a black/white CCD-chip which had a resolution of 512 pixels (horizontal) and 582 (vertical). This resolution was adequate for this purpose if the observation area on the bee hive did not exceed 10x10 cm. The general sensitivity for visible light was rated at 0.2 Lux. However we utilized mainly its extended sensivity into the infra-red region with the help of infra-red LED's. The optical lens had a focal length of only 4.48 mm, so that closeup pictures (up to 2x2 cm) were possible. The camera was connected to the controller, a monitor and a video recorder assembled in a seperate room.

Firstly, we conducted experiments to observe general defense traits of this species against *Varroa* by taking infra-red photographs in order to have their impression as reported earlier (Peng et al., 1987). For this purpose the cage having marked bees was placed in front of the camera.

Secondly, to check the genetic basis of these traits, 24 hours before emergence, the brood frames from the six colonies having different percentages of damaged mites under field conditions were placed in the incubator (34.5ºC, 50-60% RH) separately. Percentages of damaged mites falling on the bottom board is considered as a general trait to determine *Varroa* resistance (Ruttner and Hänel, 1992; Bienefeld, 1996a; Szabo et al., 1996). Twenty-four hours before the test 50 newly emerged workers from each colony were marked with numbered plastic labels of the "Opalithplättchen" type. The freshly capped cells were mite infested by following the method of Rath and Dresher (1990). We used phoretic mites freshly collected by dusting powder sugar on bees collected from a mite-infested colony in small cages. Mites dropped after gentle shaking. The sugar was removed from mites by putting them between two slightly damp filter papers. The brood frame having artificially infested cells was placed in the original unit for natural resealing. This experiment was repeated once. The whole process was video-recorded continuously for 6 days in each experiment. The marked bees (n=25) in each replication were individually infested to notice the extent of grooming and their behavior against mites. This was conducted three times in each experiment. The mites expelled or damaged were collected in the tray containing glycol at hourly intervals up to seven hours. Each fallen mite was examined under stereomicroscope for any damage. Probable ant attack on the mites was excluded by putting an oil barrier at the base of the stand.

Observations on the defense behaviour of individual bees *e. g.* removal of mites from brood, self grooming, nest mate grooming, grooming dance, bees' reaction to mites, uncapping and removal of artificially mite-infested brood were video-recorded. Data were computed after analyzing video-recordings for the purpose of obtaining duration and intensity of a trait expressed by individuals. Data were subjected to statistical analysis by using SAS (1988) procedures. The relationship between uncapping and removal, uncapping and grooming and removal and grooming was estimated by Kendall's Tau-b correlation-coefficient.

Results

Bees detected mites moving on brood frames and developing larvae. They caught the mites tightly in their mandibles with the help of their forelegs. They also used their second pair of legs when mites tried to escape. Bees holding the mites in their mandibles usually stood up on their hind legs, thereby reflecting their upright position. Before dropping, they bit and chewed the mites vigorously (Fig. 1, 2, 5). The infested bees performed self cleaning with the help of their fore and hind legs for removing mites attached to their thorax and between their head and thorax (Fig. 4a, 4b).

The bees, after getting mite infestations, started, self-cleaning and thereafter performed a grooming dance. They made vigorous lateral abdominal movements for attracting groomers. It has been observed that bees performing a grooming dance slowly, normally do not attract nest mate groomers. However, infested bees performing a fast and vigorous grooming dance after being mite infested attracted groomers immediately (Fig. 7, 7a). The group cleaning, to the maximum of two groomers, has been observed rarely. The groomers made a lot of searching movements on the body of the infested bee, which resulted in dislodging of the mite or changing its host. During this process the the infested bee stood calmly, stretching her wings. No direct removal of the mites was observed from the infested bees (n=185). However, rarely, repeated groomers' attacks on the mites attached to a bee's body were noticed, resulting in the dislodging or shifting the parasite to another host or within the same host *i.e.* hiding on the ventral surface. Grooming on the ventral surface resulted in the movement of the mite to the dorsal surface, thereby escaping from the searching and attacks of the allogroomer.

Analysis of recordings indicated that in total 30 % (6/20) and 26.6 % (4/15) percent infested cells were uncapped in the first and second experiment (Fig. A). It was noticed that in both experiments the cell opening commences on the 4th day after infestation. Data showed large variation in percentage of bees involved in the opening of mite-infested cells. Most of the bees did not participate in uncapping. Interestingly, some bees were found to be extraordinarily active in this process. One of them was even involved in uncapping of 5 of the 6 cells in first experiment. However, percentages of marked bees involved in the process of uncapping no cell, 1, 2, 3, 4 and 5 cells followed a decreasing trend in both the experiments (Fig. A).

Data also showed that in total, a high percentage of bees from colony 1 and 2 were found to be involved in uncapping of most of the cells, whereas bees from colony 6 were found to be least active in performing the above job. Similar results were obtained in both experiments (Fig. B). A high percentage of marked bees performing well in uncapping of most of the cells were from colonies 1&2, having a maximum number of damaged mites (>40%) under field conditions, in comparison to other colonies except one. On the other hand, percentage of bees (<20%) showing less activity w.r.t uncapping of mite-infested cells were from colonies having fewer damaged mites (<20%) with one exception. From the colonies having a high mite damage rate (>40%) under field conditions, a higher percentage of bees acted as allogroomers (>20%) of infested bees and vice-versa. The correlation-coefficient (r) between percentages of damaged mites under field conditions and uncapping, removing and grooming were found to be 0.63 ($p=0.07$, $n=9$), 0.43 ($p=0.25$, $n=9$) and 0.72 ($p=0.11$, $n=6$) respectively. In the first experiment with older bees (more than two weeks old) we did not find even a single event of grooming in all six colonies (Fig. C)

Data revealed significantly positive correlation ($r=0.37$, $p=0.0001$) between uncapping and removal of mite-infested cells. A non-significant correlation ($r=0.03$, $p=0.5146$) was found between uncapping and grooming behavior of marked bees and also between removal and grooming ($r=0.01$, $p=0.8179$). The recovery of damaged mites in glycol was found to be 9 (damaged, 6; expelled, 58; infested, 150) and 15 (damaged, 3; expelled, 20; infested 75) percent, respectively, in the first, and second experiment.

Discussion

The method of using Infra-red photography to study *Varroa* defense mechanisms in honey bees was found very useful for observing processes of uncapping and removal of artificially mite-

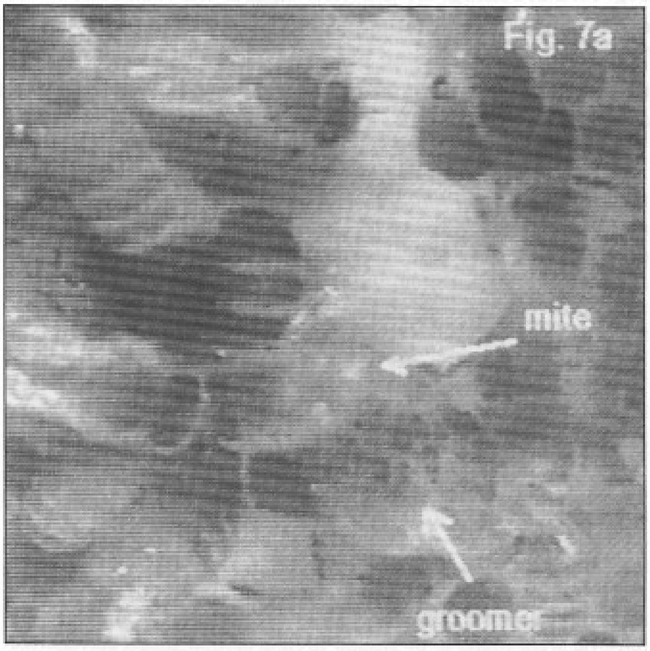

Fig. 7a. A dancing bee has stretched its wings and is sitting calmly, being groomed by a nest mate (nest mate grooming).

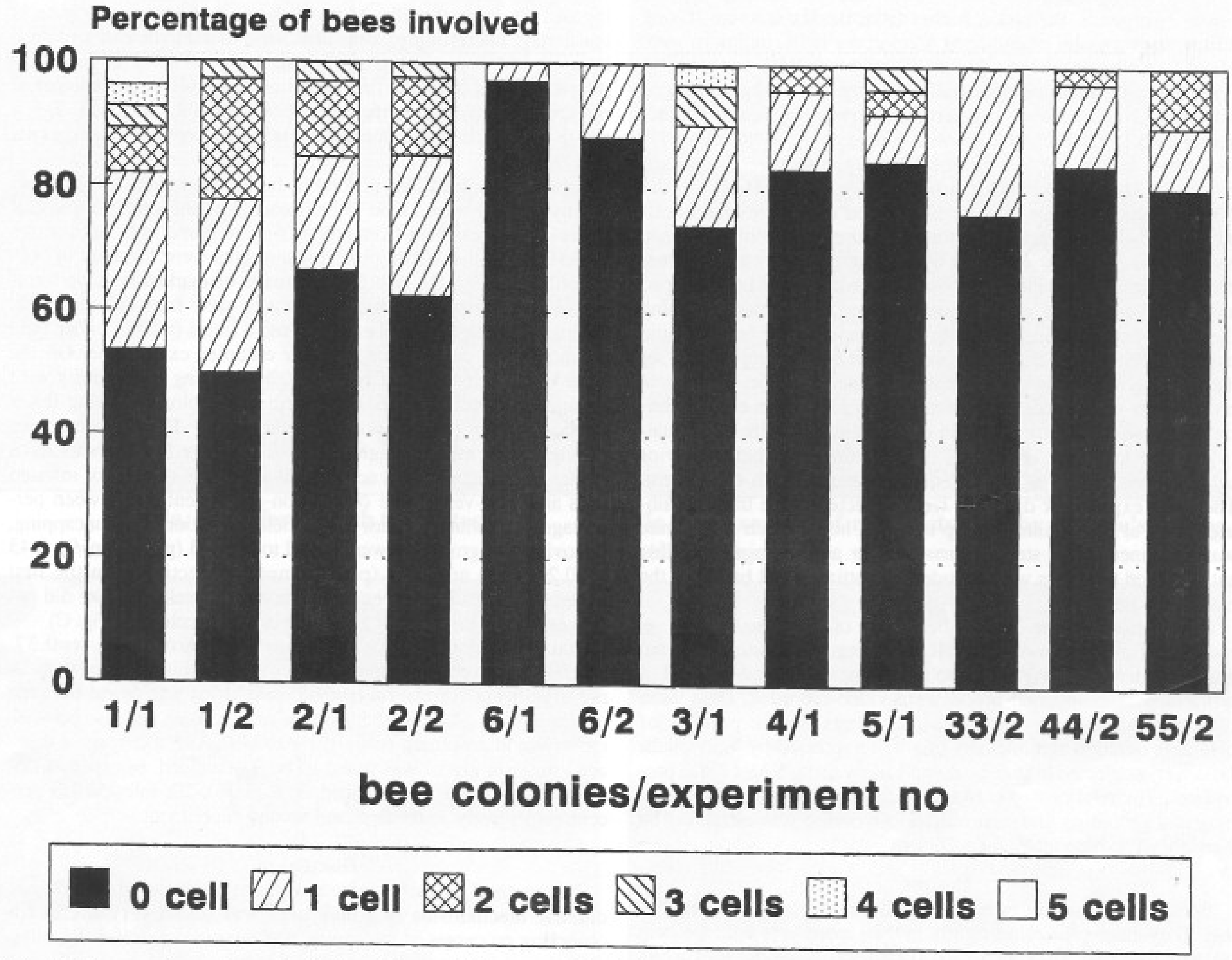

Fig. A. Percentage of marked bees from different colonies involved in the uncapping of artificially mite-infested cells in two experiments. X-axis represents numbers of the colonies being tested in first and second experiment. The differently marked areas of the columns means the percentage of bees/colony involved in uncapping of none (0 cell), 1 .... to 5 cells (of altogether 6 cells opened) in experiment 1 and none (0 cell), 1.... to 3 cells (of altogether 4 cells opened) in experiment 2, respectively.

infested cells, the reaction of individually mite-infested bees and their groomers. Infra-red photographs taken during long-term observations clearly indicated the defensive ability of *Apis mellifera carnica e.g.* detecting, biting and chewing free moving mites which provides answers to the previous assumptions and doubts regarding direct damage and removal of mites (Peng et al., 1987; Boeking and Dresher, 1991; Ruttner and Hänel, 1992; Büchler, 1994, Bozic and Valentincic, 1995). However, the extent of this kind of direct damage and its relative importance needs to be studied in detail. Confirming the results of Büchler et al., (1992) and Bozic and Valentincic (1995), during long-term analysis of recordings, not even a single allogroomer was able to remove a mite from another bee. However, rarely repeated attacks on the mites were observed resulting in dislodging, disturbing the mites and causing them to change their host. Similarly, the nest mate cleaning by two mates was rare and in the majority of the cases it was by one allogroomer. Other behaviors like self cleaning, dancing and mite removal were found to be similar to *Apis cerana* which corroborate the findings of Peng et al., (1987) regarding mode of expression of these traits. The response of uncapping and removal of artificially mite-infested cells to the extent 30 and 26.6 percent in two experiments corroborate the earlier findings of 4.8% to 100% till 10 days after infestation (Boecking and Dresher, 1991). We found no grooming activity of marked bees in the first experiment which may be because of the older bees (>14 days) which were used for these studies and indicating some age dependence for the expression of this trait. This may also be attributed to the fact that with our technique we had access to the reactions of only 60% of the marked bees performing grooming. However, all bees had equal chance to express this trait.

Significantly positive correlation between uncapping and removal of *Varroa*-infested cells indicated that both these traits are controlled partly genetically. Our results corroborate the findings of Milne (1982) who reported significant correlation while finding a relationship between uncapping and removal of freeze-killed brood. Milne (1985) estimated heritability of uncapping and removal of freeze-killed brood and also a positive genetic correlation between them which may support our results indicating common genetic control of uncapping and removal of *Varroa*-infested brood.

Our studies indicate that a substantial proportion of artificially inocculated mites got expelled. The proportion of damaged mites (9 & 15%) obtained in the expelled mites after 7 hours confirms the results of Fries et al., (1996) of 12.3% damaged mites being obtained after infesting full sized colonies. The difference in mite

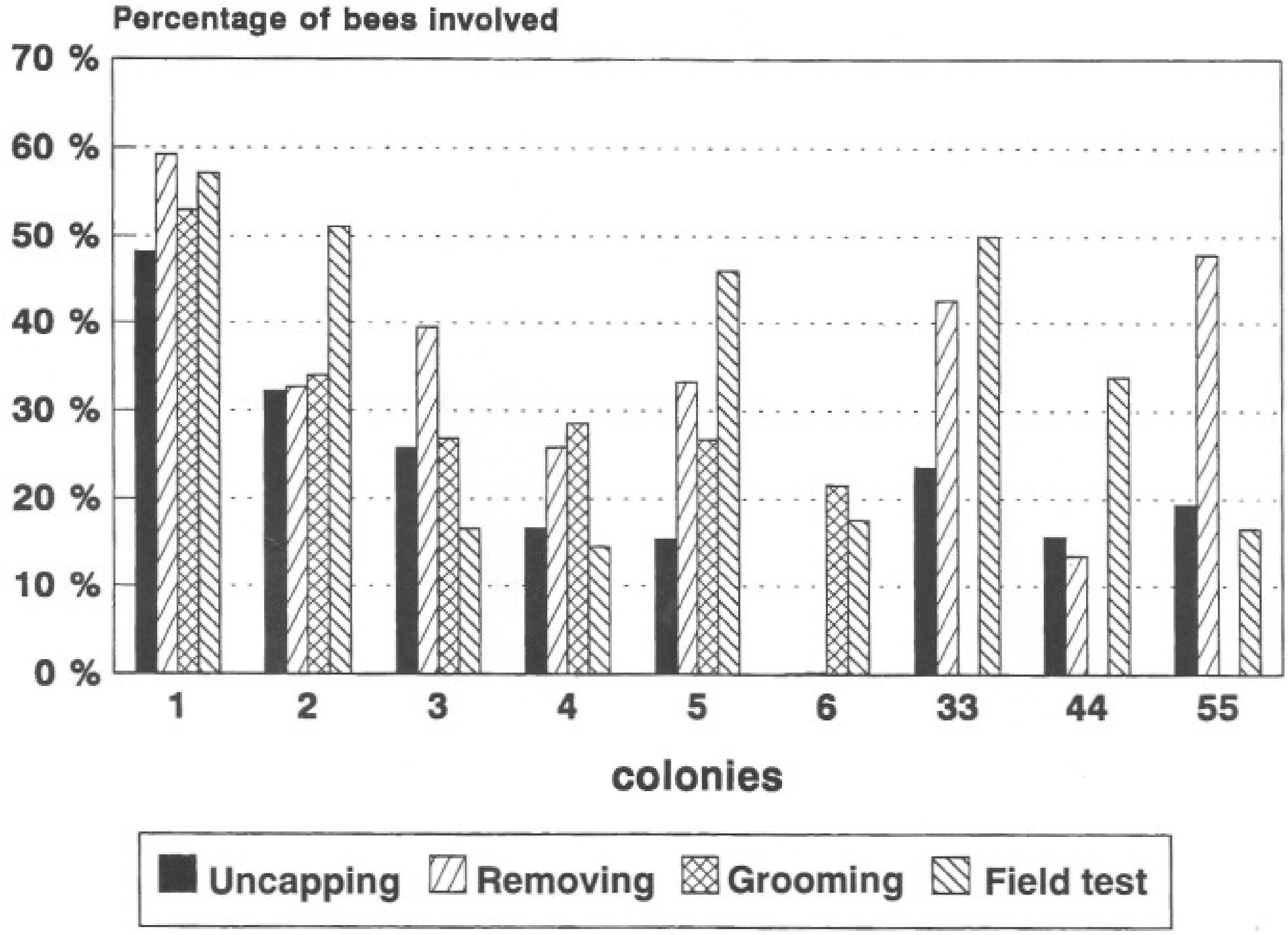

Fig. B. Relationship between field test of the colonies *i.e.* percentage of damaged mites and performance of their individuals in the process of uncapping, removing and grooming behavior during tests.

expulsion in two experiments may be attributed to the fact that in first experiment the tests were conducted with comparatively older bees that were confined for a longer time in the experimental cage and possibly the mites had the tendency to leave the host. Based upon our observations, mites are also damaged when moving on the frames or taken out of cells which showed that damage to mites should not only be attributed to the grooming behavior of bees.

Significant differences with respect to uncapping, removing and grooming and similarity of three colonies in two experiments indicated a genetic basis of these traits. However, the large variation within the colonies can limit the selection, because even in the superior grooming colonies 1 and 2, 40% and 50% of the bees, respectively, did nothing. This may limit selection response of the breeding schemes if the colony as a whole—is considered a unit. In addition, a strong age-dependent expression of some *Varroa* defense traits is also reflected (Fig. C, and unpublished results). This may pose difficulty when comparing colonies even from the same genotype, but having different age structures.

Some of the individuals performed extraordinarily during whole experiments, resulting in sufficient repeatability of some of the *Varroa* defense traits (Thakur et al., 1996b). Such outstanding individual behavior indicates that it is pertinent to make use of these individuals. We have started using individuals expressing exceptional grooming by stimulating them to lay eggs and further using them for breeding *Varroa*-tolerant lines (unpublished results).

This method is not practical in normal breeding programs supported by beekeepers because it involves scientific techniques. The extent of mite damage under field conditions was found to be related with performance of the individuals in our tests. However, in the present studies, we evaluated only nine colonies. We intend to confirm this positive relationship by testing individuals from more colonies having different percentages of damaged mites in full colonies in the field. However, positive correlations between damaged mites in the colonies and their individual bee performance in expression of defense traits corroborate earlier studies indicating mite damage as a useful tool and a very desirable characteristic for identifying *Varroa*-tolerant colonies (Bienefeld, 1996a; Szabo et al., 1996), provided one can optimize sampling timing and technique (Bienefeld, 1996b).

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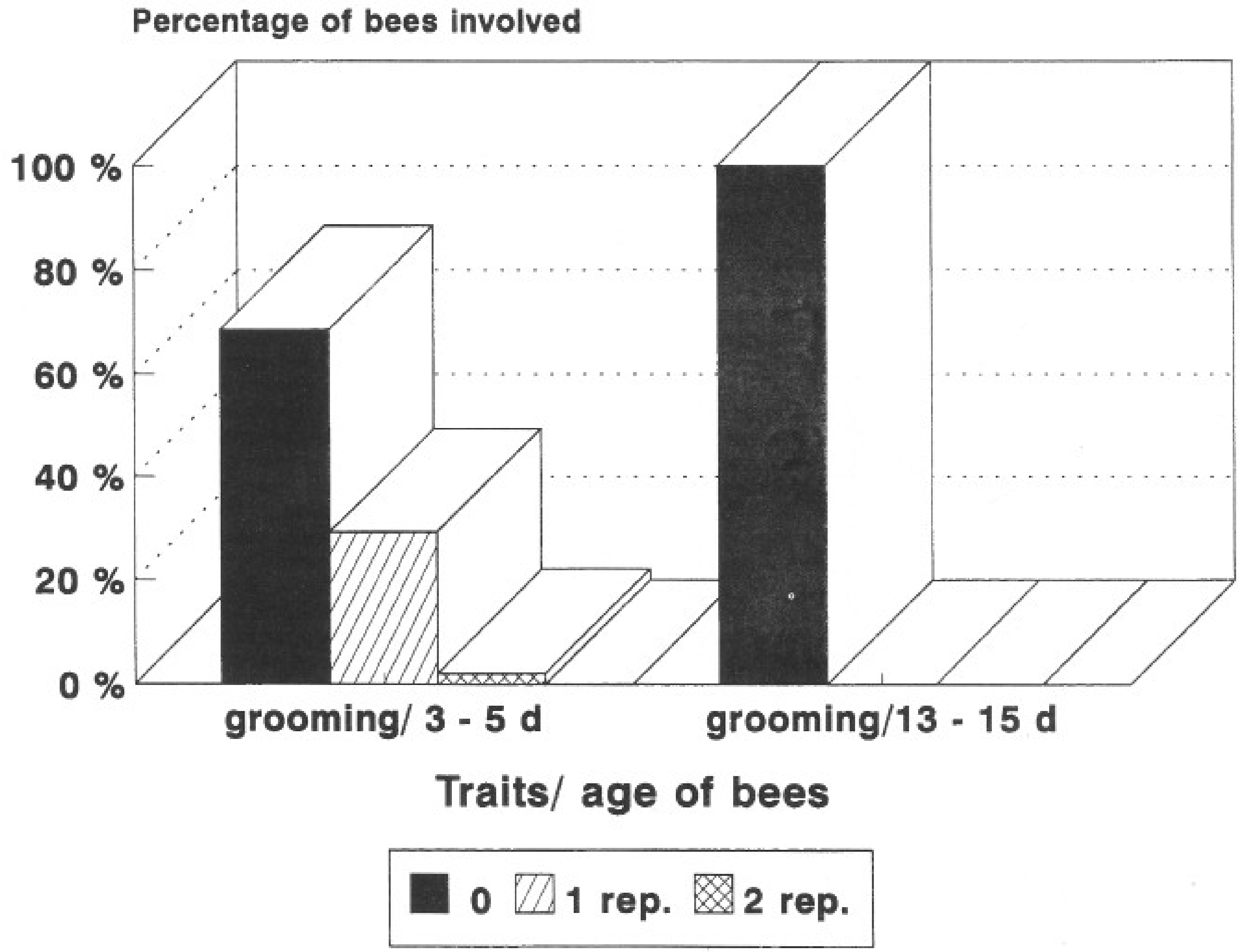

Fig. C. Performance of different aged bees with respect to grooming in three replications of first and second experiment. Columns represent percentage of bees involved in no grooming (0), only in one replication, in both 1 and 2 replications of second experiment. Not even a single bee (1. experiment, bees were older than 2 weeks) participated in all three replications.

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