Chapter 19

Defence Behaviour of *Apis mellifera carnica* against *Varroa* and Inheritance of Defence Traits

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Beekeeping with *Apis mellifera* is now endangered world-wide by the mite, *Varroa jacobsoni*. *Varroa* can be controlled chemically but this has resulted in the contamination of hive products (Boeking and Ritter, 1994). Recently reported acaricide-resistant strains (Milani and Vedova, 1996) and limitations of chemical control have led scientists to explore the possibility of selecting *Varroa*-resistant strains. Some studies have indicated the natural defence mechanism in *A. mellifera* (Büchler *et al.*, 1992; Peng *et al.*, 1987; Ruttner and Hänel, 1992). Direct evidence regarding the defensive abilities of *A. mellifera camica* against *Varroa* in the form of video observations and infra-red photographs have been reported (Boeking, 1994; Thakur *et al.*, 1997). *Varroa*-defence is normally measured indirectly by per centages of damaged mites falling on to the bottom of hives (Bienefeld, 1996; Büchler, 1994) or the removal of artificially killed brood (Hoffman, 1996). Both methods are strongly affected by environmental influences. Colony-level selection may not be particularly efficient for *Varroa*-resistance breeding because of significant intracolonial variations. Thakur *et al.* (1997) found that *Varroa*-resistant traits, e.g, uncapping and removing, are performed at various levels within a colony. Some workers were found to be extremely active, whereas the majority were inactive. Boeking (1994) observed several bees repeatedly removing cell cappings. These results may be explained by the fact that colony composition has various patrilines with a different distribution of behavioural response thresholds for stimuli eliciting a task.

However, the different behaviour levels with respect to uncapping and removal of *Varroa*-infested cells may be caused by environmental effects. To quantify the inheritance of this *Varroa*-tolerance behaviour, these investigations were designed with the use of honeybee reproduction in particular. Moreover, breeding from workers may have advantages (Boeger, 1969) because it may provide the precision needed to detect genetic combinations that occur at low frequencies such as resistance to *Varroa* (Harris and Harbo, 1991).

Materials and Methods

Twenty-four hours before emergence, brood frames from six colonies with different per centages of damaged mites were separately placed in an incubator (34.5°C, 50–60% R.H.).

Workers (n = 50, 0–12 h old) from each colony were labelled with numbered plates on the thorax. Marked bees were maintained in a frame cage with a sliding panel of glass on one side and metal gauze on the opposite side. The cages, with four bee frames and one adjacent brood frame, were placed in a polystyrene mating box. An infra-red video camera was placed in front of the glass panel (Thakur *et al.*, 1996).

Marked workers were evaluated for selection of individuals that performed excellently during uncapping and removal of artificially mite-infested worker brood and responded to mite-infested dancers by nest-mate grooming. Mites were introduced in freshly capped worker brood (Rath and Drescher, 1990). Grooming behaviour and reactions of workers individually infested with mites was recorded continuously. Some excellently performing workers (n = 15) were maintained in a queenless and broodless nucleus containing young bees and sufficient provisions. Unmarked workers were replaced by a new batch of newly emerged workers at 5–8-day intervals (Harris and Harbo, 1988). Drone-rearing and drone-holding units were continuously supplied with pollen.

Queens (n = 5), reared from a homogenous stock of *A. mellifera carnica*, were instrumentally inseminated with mixed semen (3–4 µl) from drones (n = 8–12 each) reared from selected laying workers. Progeny from one cross was evaluated for genetic gain with regard to uncapping of artificially *Varroa*-infested brood, removal of uncapped cells and nest-mate grooming. The test population of the progeny (55 bees) was compared against the control (95 bees). The control consisted of bees taken from a well-established and homogeneous line from which the queens were reared.

Varroa-defence responses of marked bees in both the selection and progeny testing experiments were video-recorded continuously for seven days using an infra-red photography technique (Thakur *et al.*, 1996, 1997). Data were subjected to non-parametric analysis by using a Chi-square test (SAS Institute, 1988).

Results

With the control set at 100%, performances of bees supplying semen for the production of the next generation were 894% for uncapping, 333 % for removing and 54% for nest-mate grooming (Fig. 1). In total, 53.6% of the control and 10.9 % of the bees from Fl were not found to be involved in uncapping of 11 artificially *Varroa*-infested cells. Of the selected offspring, 25.4% were involved in the uncapping of more than five infested cells compared to only 6.3% of control bees. Uncapping of eight of the 11 infested cells was initiated by bees from the F1 generation (Fig. 2).

Behaviour concerning removal was also similar but less distinctive. The frequency of Fl bees involved in the process of removing was also higher in comparison to the control. With the control set at 100%, the performances of the offspring were calculated to be 290%, 239% and 157%. The uncapping process began between 4–5 days after infestation.

Discussion

Present studies show genetic improvement with regard to uncapping and removal of artificially *Varroa*-infested brood since a higher per centage of bees from Fl were found to be involved in comparison to the control (Fig. 3). Moreover, in 72.7% (8/11) of the infested cells, the uncapping process was started by bees from the Fl generation, which indicates that more of them were capable of detecting mites in the brood cells, possibly by perceiving signals, in comparison to bees from the control.

The fact that the uncapping of most of the *Varroa*-infested cells began between 4-5 days after infestation (Fig. 4) indicates that disturbance by the mite caused to the growing pupa resulted in the release of some cues or signals. These cues were possibly perceived by the uncappers and resulted in the initiation of opening. This is also supported by the fact that when freshly capped brood cells are infested and returned to a colony,

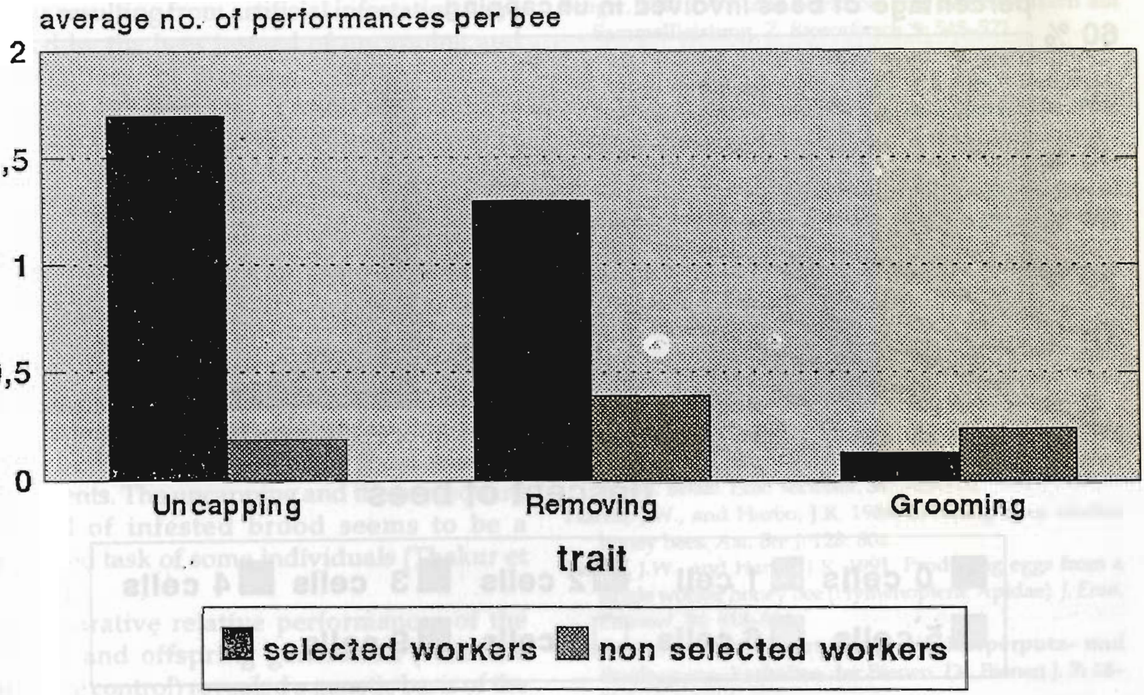

Fig. 1 Average performance of selected and non-selected workers

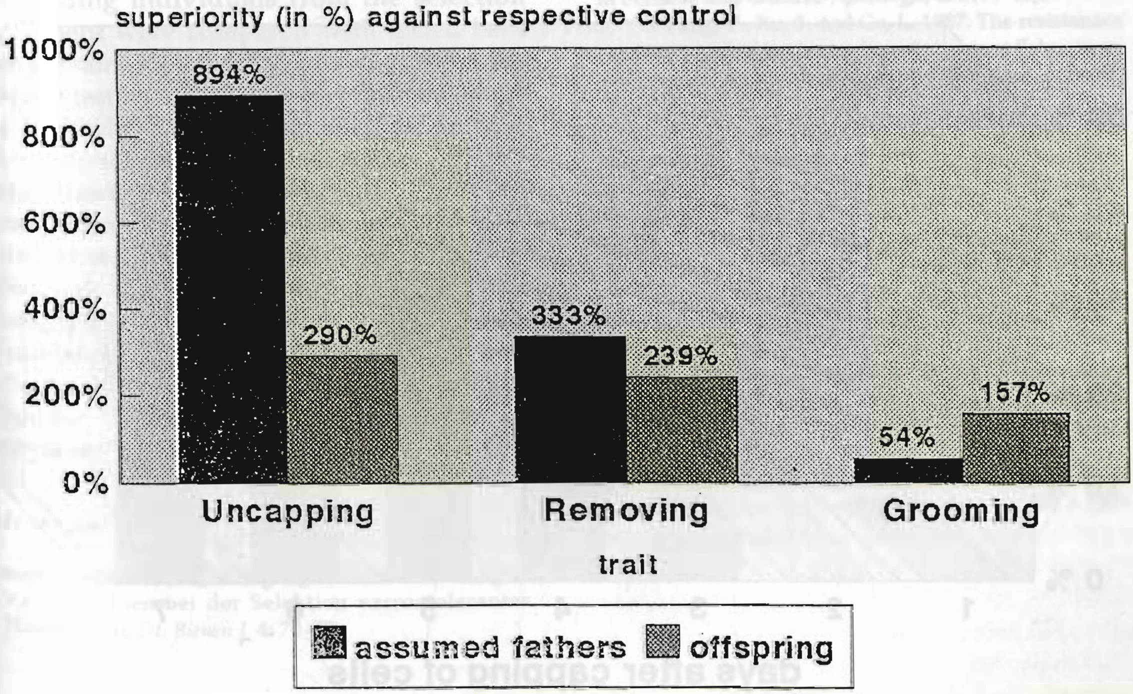

Fig. 2. Percentage superiority of assumed fathers and offsprings of assumed fathers

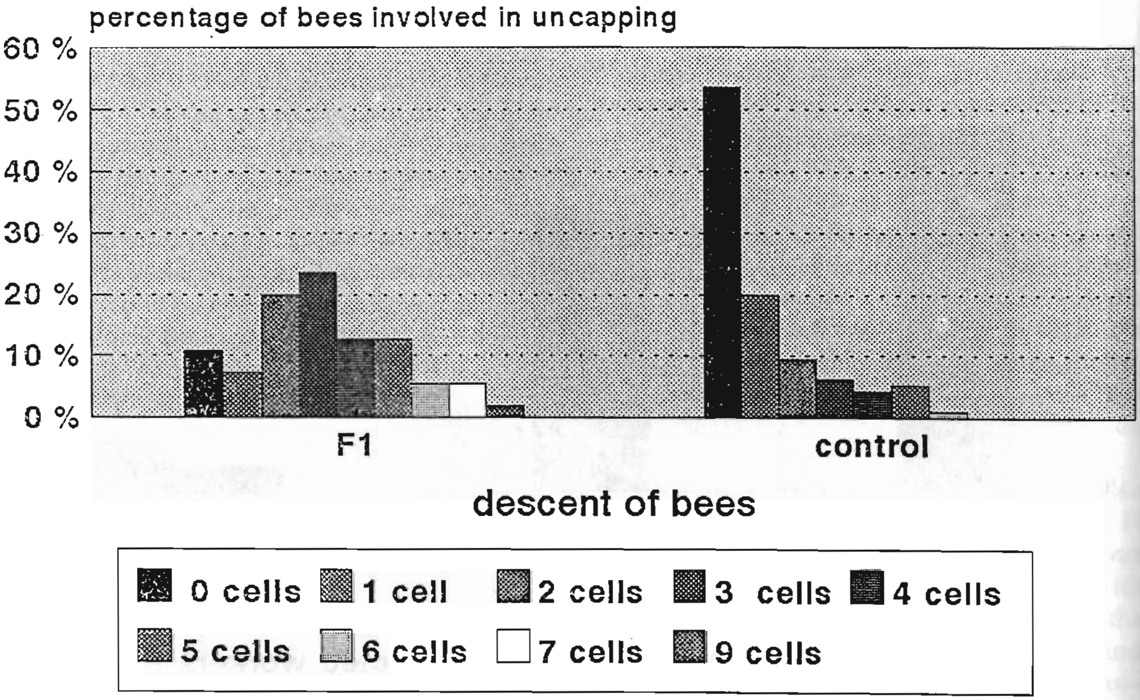

Fig. 3. Percentage of workers involved in uncapping

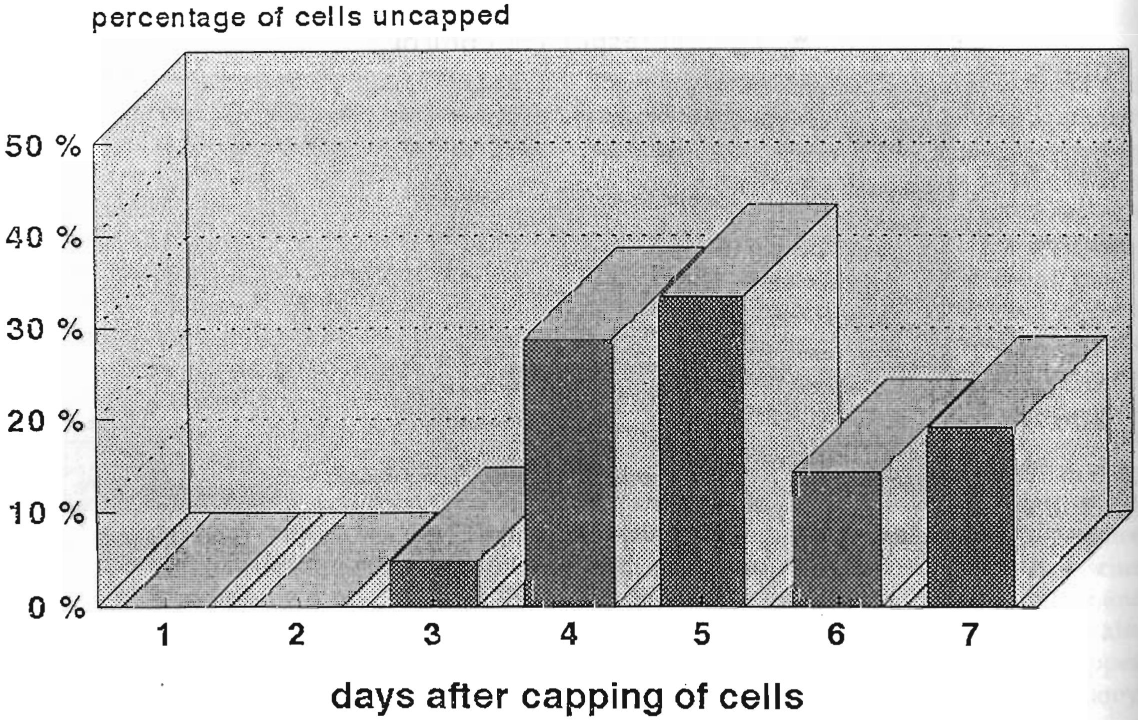

Fig. 4. Time of uncapping

the cuts resulting from artificial infestation were resealed by the bees instead of uncapping and removing larva at that stage. The initiation of uncapping between Day 4 and Day 5 may be caused by the rhythmic behavioural activity of mites searching for a feeding site on the pupal body (Donze and Guerin, 1994). The rise in these activities may cause the release of certain stimuli or cues by pre-pupae and pupae because of injuries or irritation as a result of feeding mites. These cues may be perceived by some specialists which in turn start uncapping. The possible preference of only a few bees to respond to the above stimuli and start the uncapping has a genetic basis as demonstrated by these selection experiments. The uncapping and the consequent removal of infested brood seems to be a specialised task of some individuals (Thakur et al., 1997).

Comparative relative performances of the parental and offspring generation (with the respective control) revealed a genetic basis of the traits for uncapping and removing. However, controls are not directly comparable because the outstanding individuals from the selection experiment were compared with tested bees from the same six lines. The control group in the progeny testing experiment were from a single line from where the mothers of all control and tested individuals came. These findings revealed partial transfer of superiority with respect to uncapping and removal behaviour to the next generation. This procedure of selecting individuals for *Varroa*-defence traits – making drone layers and using the resulting drones to inseminate the queens of selected stock – seems to be an efficient approach. It may help in inducing *Varroa*-tolerance/resistance in honeybees.

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