The Gentleness of *Apis dorsata* Verified while Investigating Brood Cross-Fostering and Hygienic Behavior

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ABSTRACT

The studies were conducted in Puna and Bangalore, India and in Rampur, Nepal. Different ways of working *A. dorsata* nests were investigated. Cross-fostering of brood of other species was conducted in order to recognize the cues used by *A. dorsata* in the recognition and acceptance of bees. Hygienic behavior was also investigated. The results show that the use of little smoke as well as slow movements was essential in working with *A. dorsata*. The authors were able to work *A. dorsata* nests without any protection, even without a shirt on. It was found that *A. dorsata* workers rejected *A. mellifera, A. cerana* and *A. laboriosa* workers which emerged from their nest despite their having been familiarized there as brood for several days. It was concluded that *A. dorsata* uses genetic differences between bees to recognize and reject other species. *A. dorsata* does not open sealed cells with dead brood infested by mites. Thus, migration does not eliminate mites left in sealed cells behind, since those cells would not have been opened even if the colonies did not migrate. Not opening the sealed cells with dead brood prevents the spread of diseases and mites. This type of hygienic behavior may be more efficient than opening cells with dead brood which is performed by *A. mellifera.*

INTRODUCTION

The extraordinary defense behavior of *Apis dorsata* is well known. Roepke (1930) described an *A. dorsata* attack, during which the worker bees followed him for 500 m. When Lindauer (1956) was attacked, the bees followed him for 2 km. Near Anuradhapura, Sri Lanka, three water buffalos were killed by *A. dorsata*. According to Morse and Laigo (1969) there is no question that this is the most ferocious stinging insect on earth. Their experience indicates that while it is probably not possible to work *A. dorsata* nests without receiving stings, the number may be minimized by using certain precautions. Therefore, they used a heavy suit of aluminized rayon and observed the colonies from inside wooden, screen-covered cages (90 x 90 x 180 cm). The authors tried different methods of working *A. dorsata* nests to be able to conduct investigations on the species.

An investigation of the cross-fostering of brood from phylogenetically distant bee species can reveal the cues used by the bees in the recognition and acceptance of other species, including both those of genetic origin, as well as those acquired by introduced species in the nest of the new host. This will enable the study of bee behavior under changed conditions (Woyke et al, 2004).

The hygienic behavior of honey bees is an important phenomenon in the resistance against diseases and parasitic mites. There are suggestions that this aspect may differ between *A. mellifera* and *A. dorsata*. In *A. mellifera,* the opening of sealed cells with infested brood within few days is considered hygienic (Spivak and Gilliam, 1998). On the other hand, Woyke (1984, 1996) suggested that *A. dorsata* workers do not open

sealed cells with brood killed by *Tropilaelaps clareae*. Koeniger et al. (1993, 2002) found a number of capped cells in combs left by colonies, which migrated. They suggest that leaving behind mites in sealed cells may represent a mechanism of mite elimination.

MATERIAL AND METHODS

The investigations were conducted in Puna, India in 1974, in Rampur and Bharatpur, Chitwan, Nepal in 1999/2000, and in Bangalore, India in 2002. *A. dorsata* nests in Puna were located in five three-wall hives constructed by Thakar (1973). In Nepal and India, the nests hanged freely from buildings or water tank towers. Although many nests were present, the authors worked directly on 15 nests in Nepal and 4 in India.

Different methods were tried in approaching *A. dorsata* colonies to cut pieces of combs from the nests. The nests were approached either without smoke and any protection, or wearing a protective apron and bee veil. The authors also used different amounts of smoke and approached the nests in bee veil, without any head protection, and without any shirt.

The acceptance of *A. mellifera*, *A. cerana* and *A. laboriosa* brood by *A. dorsata* was investigated in Nepal. For that purpose, three pieces of sealed brood combs with a width of 10 x 10 cm were cut out from *A. mellifera* colonies. In addition, three pieces of sealed brood combs 10 x 8 cm in width were cut out from *A. cerana* colonies. In the case of *A. laboriosa,* only one piece 15 x 10 cm in width was used. Next, appropriate holes were cut out in the brood area of seven *A. dorsata* combs, into which the three *A. mellifera,* three *A. cerana* and one *A. laboriosa* brood pieces were inserted. The nests were inspected daily. Also every 10 *A. mellifera* workers emerging in an incubator were put in five small wire mesh cages with dimensions of 9 x 6 x 1 cm. The cages with bees were introduced into five *A. dorsata* nests. This was repeated three times. The number of survivors was checked.

The hygienic behavior of *Apis dorsata* was investigated in Bangalore, India. Three small comb pieces with 120 to 369 sealed pupae were cut from three *A. dorsata* nests. They were frozen for 24 hours at -20°C, and then reintroduced into the same nests. The killed brood was checked daily. The number of pupae left and the nature of cell capping on brood cells was examined.

All of these procedures are presented on video and described below.

RESULTS

Approaching *Apis dorsata* nests

In Poona, Dr. J. Woyke approached the five *A. dorsata* nests in the three walled hives without the use of smoke and without any protection. It was essential to approach the colonies very slowly. When the author was breathing in the direction of the nest curtain, the workers started to fly to his direction and sting him. However, when he refrained from breathing, he was able to slide the curtain bees and observe the combs.

In Nepal, when the ambient temperature was below 15°C, *A. dorsata* workers did not fly, and when temperature was below 12°C they were chilled and it was possible to work the nests without any protection. When the temperature in Nepal and India was above 17°C, however, the bees were flying. However, the authors were able to approach the nest without any smoke or protection to a distance of 0.5-1.0 m. It was

necessary to approach the nests and perform any movements very slowly. Any violent movement provoked the workers to fly and sting. The reaction of *A. dorsata* to smoke pointed in the direction of the nest was different. Much smoke provoked the bees to fly in a cloud and attack. On the other hand, less smoke caused fewer bees to fly. Gentle smoke of 2 to 3 puffs of the smoker, provoked only a few bees to fly and these returned to the nest after a while. Afterwards, the bees remained calm. When the authors again smoked the nest a little, the bees moved, clearing the area around. This allowed the authors to work the combs without any head protection or shirt, and observe the content of the combs. They were also able to cut pieces of combs from the nests and introduce pieces of brood from other bee species, as well as investigate the effect of brood cross-fostering. Frozen brood was also introduced to examine the hygienic behavior of *A. dorsata*.

Introduction of brood from other species into *A. dorsata* nests

The examination of *A. mellifera* brood introduced into *A. dorsata* nests did not reveal any damage to the cappings. These were not damaged even nine days after introduction. However, young *A. mellifera* workers, which emerged in the presence of the authors, were molested by *A. dorsata* workers. As a result, both or only *A. mellifera* worker dropped down from the combs.

The inspection of *A. cerana* combs inserted into *A. dorsata* nests showed that some empty small *A. cerana* comb cells were reconstructed into large *A. dorsata* cells. However, cappings on sealed *A. cerana* brood cells were not damaged. Nevertheless, the small *A. cerana* cappings were reconstructed into large *A. dorsata* cappings. As a result, three *A. cerana* brood cells were covered by one large *A. dorsata* cap. After *A. cerana* workers emerged, however, they were molested by *A. dorsata* bees and then dropped out of the nests.

Cappings on cells with *A. laboriosa* brood in *A. dorsata* nests were not destroyed either. However, *A. dorsata* did not accept emerged *A. laboriosa* worker bees. A few days after emerging, none of the worker bees of the three species was found in *A. dorsata* nests. While *A. dorsata* did not damage sealed brood of the other bee species, it did not accept emerging bees of those species even when the brood were in the nests for 10 days. An examination of caged *A. mellifera* workers in five *A. dorsata* nests revealed that no bee was alive on the second day after introduction, in all tree repetitions.

Brood hygienic behavior

The day after freeze-killed *A. dorsata* brood were inserted into *A. dorsata* colonies, pupae were found to have been removed from all physically damaged cells along the edges of the inserts. There still remained 100 to 310 sealed pupae per freeze-killed replicate. After 4 days, 95% of pupae remained in the colonies. Cappings on all cells remained intact until the end of the observation period. The cells were not opened by the bees even after all of the workers had emerged from the brood area around the insert. This shows that *A. dorsata* workers do not open sealed cells containing dead brood.

DISCUSSION

The results show that by applying appropriate procedures, it is possible to work *A. dorsata* nests without any protection and even without a shirt. The possibility of easily working the nests allowed the authors to study the recognition cues used to accept or reject other bee species, as well as to study the hygienic behavior of the species.

A. dorsata did not damage unfamiliarized *A. mellifera, A. cerana, or A. laboriosa* brood introduced into their nests. However, *A. dorsata* did not accept young workers of those species, despite the fact that they emerged from *A. dorsata* nests and were familiarized to the colonies, having been in them as brood for several days. *A. dorsata* also did not take care of young *A. mellifera* workers introduced into the nests in small cages. Thus, it is evident that *A. dorsata* does not use environmental cues acquired by the introduced bee species in *A. dorsata* colonies. Instead, it uses genetic cues in the recognition and rejection of other bee species.

The results show that *A. dorsata* does not open cells with dead brood. Therefore, mites cannot escape from infected cells with dead pupae. This indicates that migration does not eliminate mites left behind in deserted combs in sealed cells with dead brood. Those cells would not have been opened, and the mites would not have come out, even if the bees did not migrate. However, the migration of *A. dorsata* indirectly eliminates large numbers of mites due to a lack of the brood in the colonies shortly before they leave the combs. Since *T. clareae* cannot feed on adult bees (Woyke 1984 1985), the mites must die shortly before migration. Both experiments show that *A. dorsata* does not open sealed cells containing unusual (dead or other species) brood. This hygienic behavior of not opening cells with dead brood reduces the spread of diseases and mites. It decreases bee diseases or mite populations more effectively than the opening of infected brood cells by *A. mellifera* or *A. cerana.*

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