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TYPE OF RECOGNITION CUE AS A BASIS TO ELABORATE A METHOD TO KEEP TWO PHYLOGENETICALLY DISTANT HONEY BEE SPECIES *APIS DORSATA* AND *APIS CERANA* IN THE SAME COLONY

JERZY WOYKE[1], JERZY WILDE[2] and MARIA WILDE[3]

[1]Agricultural University, Apiculture Division, 166 Nowoursynowska, 02-787 Warsaw, Poland, [2]WM University, Apiculture Division, Olsztyn, Poland

[3]Dabur Apicultural Centre, Jugedi, Chitwan, Nepal.

SUMMARY

The purpose of the investigation was to elaborate a method to keep A. dorsata bees in A. cerana colonies. This would enable to study behaviour of A. dorsata in changed environmental conditions. To achieve this, recognition cues were studied, which A. cerana and A. dorsata use in acceptance or ejection of the other species. The investigations were conducted in Chitwan, Nepal. Pieces of A. dorsata brood were introduced into 5 A. cerana nests and pieces of A. cerana brood were introduced into 3 A. dorsata nests. The results show that A. dorsata workers, which emerged shortly after brood introduction were molested by A. cerana workers and some were ejected from the hives. A. dorsata workers which emerged on following days, and thus became familiarized, were accepted and both species of bees coexisted for the most part peacefully. The last A. dorsata worker was found in A. cerana colony 1 month after brood introduction. After A. cerana brood was introduced into A. dorsata nests, the small cappings on sealed A. cerana brood cells were reconstructed into large A. dorsata cappings. A. cerana workers which had emerged were molested by A. dorsata bees, and dropped down out of the nests. Thus, the results show that A. cerana workers use cues acquired by the introduced A. dorsata bees in the host colonies, to accept the other species. However, A. dorsata workers use heritable differences between both species as cue to eject the other species. Hence, familiarized A. dorsata bees may be kept in A. cerana colonies.

Keywords: *Apis dorsata*, *Apis cerana*, recognition cues, acceptance of other bee species.

INTRODUCTION

Large part of honey harvested in Southeast Asian countries origin from the free-living *Apis dorsata*. Biology of that bee is not well known. Some of the reasons are the difficult access to that bee living in high altitudes and the great defensiveness. The possibility to keep *A. dorsata* bees in the hive nesting *A. cerana* colonies of different biology, would enable to detect which behavioural traits of *A. dorsata* are unchangeable and which may be modified in other living condition. Thus, learning ability of *A. dorsata* could be studied. Cross fostering of both species would enable to detect which cues, the inherited or the acquired play role in the recognition and acceptance of one species in the colony of the other one.

The ability to recognize related and unrelated, or more and less related *Apis mellifera* workers from other colonies was demonstrated by Breed (1983), Getz and Smith (1983) and Moritz and Hillesheime (1990). All the authors concluded that honey bees used genetic cues in the recognition of *A. mellifera* workers in laboratory conditions. However, Downs and Ratnieks (1991) showed that, in field conditions, *A. mellifera* guard bees use non-heritable cues in recognition of conspecifics.

Sakagami (1959), Dhaliwal and Atwal (1970) investigated heterospecific relations between two cavity nesting species, *A. mellifera* and *A. cerana*. However, Woyke *et al.* (2000, 2001 and 2004a), investigated recognition and acceptance of two phylogenically distinct species; the cavity nesting *A. mellifera* and the two free nesting *A. dorsata* and *A. laboriosa*. The purpose of this study was to elaborate a method to keep *A. dorsata* bees in the nests of the other species. To do this, cues used in recognition and acceptance or ejection of one species, by the other were investigated.

MATERIALS AND METHODS

The investigations were conducted at the Dabour Apiculture Centre in Jugedi and at the Institute of Agriculture and Animal Science of the Tribhuvan University in Rampur, Chitwan, Nepal, from December 1999 to May 2000. Five experiments were conducted, to introduce one species of bee, *A. dorsata* or *A. cerana*, into the nests of the other species. The treatment and survival of brood or adult worker bees introduced into the nests of the other species was checked daily, or periodically. Behaviour of *A. dorsata* bees at the hive entrance of *A. cerana* colonies was also observed. In order to investigate whether *A. cerana* accepts *A. dorsata* brood, two experiments were conducted:

Introduction of young *A. dorsata* brood into *A. cerana* colonies

Part of brood comb was cut from an *A. dorsata* nest. Three pieces 6 cm x 9 cm of brood were selected. The brood consisted of larvae 4 days old to pupae with red eyes. Next, three brood combs were removed from three *A. cerana* colonies. Appropriate holes were cut in the centre of each comb and *A. dorsata* brood was inserted into the holes. The combs were returned to their colonies in the centre of the nests.

Introduction of old *A. dorsata* brood into *A. cerana* colonies

A similar experiment was conducted, but with older *A. dorsata* brood, which consisted of pupae 3 days before emerging, up to emerging workers. The brood was introduced into 5 *A. cerana* colonies between January and April. In order to investigate whether *A. cerana* accepts adult *A. dorsata* workers two experiments were conducted:

Introduction of young emerged *A. dorsata* workers directly into *A. cerana* colonies

Piece of *A. dorsata* brood comb was put into an incubator. After some workers emerged, per 25 to 35 were added in the evening to three *A. cerana* colonies. Two colonies were strong and one was weak.

Introduction of emerged *A. dorsata* workers in small cages into *A. cerana* colonies

Per 10 *A. dorsata* workers which emerged in an incubator were put into each of 3 small wire mesh cages 9 cm x 6 cm x 1 cm. They were located individually in the centre of 3 *A. cerana* brood nests. In order to investigate whether *A. dorsata* accepts *A. cerana* brood, the following experiment was conducted:

Introduction of *A. cerana* brood into *A. dorsata* nests.

Brood combs were removed from *A. cerana* colonies. Three pieces measuring 10 cm x 8 cm containing larvae and sealed brood were cut out. Next, appropriate holes were cut out in the brood area of three *A. dorsata* combs. The *A. cerana* brood pieces were inserted into the three holes. The three nests and combs were inspected daily.

Behaviour of *A. dorsata* workers at hive entrances of *A. cerana* colonies

The behaviour of *A. dorsata* workers at the entrances of *A. cerana* hives was observed periodically. The behaviour of *A. cerana* guard bees towards introduced *A. dorsata* workers was also noted.

RESULTS

Introduction of *A. dorsata* brood into *A. cerana* colonies

Introduction of young *A. dorsata* brood

The next day, after the young *A. dorsata* brood was introduced into *A. cerana* colonies, open brood was not found. Sealed brood remained, however, an average of 75% cappings were scratched. Single brood cells were opened. After 2 to 6 successive days, 25%, 37% 50%, 75% and almost all cells were opened the respective days, and the brood in half of them was eaten. Partly eaten pupae remained in some cells. Within one week all the brood cells were opened and the brood was eaten.

Introduction of old *A. dorsata* brood

After *A. dorsata* brood with adult pupae was introduced in January and February into three *A. cerana* colonies, the cappings were not scratched. *A. dorsata* workers, which emerged shortly after brood introduction, were molested by *A. cerana* workers and some were ejected from the hives. During the next day 20 - 35 *A. dorsata* workers were found in the colonies. Some (2 - 3) were observed to be molested and others were left undisturbed. On the following days more *A. dorsata* workers emerged, and it is estimated that about 300 *A. dorsata* workers emerged in each colony. *A. dorsata* workers, which emerged few days after introduction of the brood, mostly were not molested and the majority of both species coexisted peacefully. In January and February, during nectar flow, only single *A. dorsata* workers were molested. In colony No 12, 10 workers were found 20 days after brood introduction, 4 workers after 28 days and the last one was found 1 month after brood introduction. In March, more *A. dorsata* bees were molested. In April, during dearth period,

even more *A. dorsata* workers were molested, and no *A. dorsata* worker survived more than one week in two *A. cerana* colonies. Thus, more *A. dorsata* workers survived for longer time in *A. cerana* colonies during nectar flow, than during the dearth period.

Introduction of adult *A. dorsata* workers into *A. cerana* colonies

Introduction of young emerged *A. dorsata* workers directly into *A. cerana* colonies

The next day after adult *A. dorsata* workers were added to *A. cerana* colonies, one or no *A. dorsata* were found in the two strong *A. cerana* colonies. In the weaker colony, out of 35 introduced *A. dorsata* bees, 30 workers were found 2 days later, 20 - 3 days later, and 5 - 1 week later. Thus, unfamiliarised adult *A. dorsata* workers were not accepted in normal *A. cerana* colonies.

Introduction of emerged *A. dorsata* workers in small cages into *A. cerana* colonies

After 3 cages, each with 10 emerged *A. dorsata* workers, were added at the end of March into 3 *A. cerana* colonies, 8, 9 and 10 workers were found alive the next day. The last survivor was found 5, 7 and 13 days later, respectively. Thus, the cage protected unfamiliarised *A. dorsata* workers from being ejected on the first day from *A. cerana* colonies. However, after *A. dorsata* bees became familiarised, *A. cerana* workers fed them for up to about two weeks (Fig. 1).

Introduction of *A. cerana* brood into *A. dorsata* nests

After *A. cerana* brood was introduced into three *A. dorsata* nests, all larvae were eaten on the first day. 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 they walked quietly between *A. dorsata* workers. However, after a while, *A. dorsata* bees molested young *A. cerana* workers, which caused that *A. cerana* workers dropped down out of the nests. Few days later no *A. cerana* workers were found in *A. dorsata* nests.

Behaviour of *A. dorsata* workers at hive entrances of *A. cerana* colonies

A. dorsata workers were flying out and returning to *A. cerana* hives. The *A. cerana* guard bees usually allowed *A. dorsata* workers to enter without any difficulties. It sometimes happened that *A. dorsata* did not return to the entrance in direct, straight flight, but in a zig-zag flight. In these cases it sometimes happened that *A. cerana* workers in the entrance and on the front wall of the hive made the characteristic abdominal side-to-side "shimmering" movements. Nevertheless, returning *A. dorsata* worker bees were allowed to enter the hive, like returning *A. cerana* foragers. Other behavioural phenomena of *A. dorsata* worker bees in *A. cerana* colonies will be published separately.

DISCUSSION

For the first time, it was possible to keep the phylogenetically distant *A. dorsata* bee species in *A. cerana* colony. The success of this enabled us to study different behavioural traits of *A. dorsata* in changed environmental conditions, which will be published later.

The results obtained showed, that normal *A. cerana* colonies do not accept neither unfamiliar brood nor young *A. dorsata* workers. Thus inherent cue of the introduced *A. dorsata* resulted in the ejection of this species in *A. cerana* colonies. However, after *A. dorsata* brood remained in *A. cerana* colonies for 1 or 2 days, emerging *A. dorsata* workers were accepted and survived for up to 1 month. Thus, familiarization of *A. dorsata* workers for 1-2 days before emerging resulted in their acceptance. *A. dorsata* workers, which emerged in an incubator and were introduced directly into *A. cerana* colonies were rejected. However, those introduced in cages were protected from being removed immediately after introduction. After short time they became familiarised, and were fed by *A. cerana* workers. As a result some survived almost 2 weeks. Thus, a short period of 1 day sufficed for familiarisation with the other species. Hence, the recognition cue acquired by *A. dorsata* bees in *A. cerana* colonies directed the acceptance of adult *A. dorsata* workers in *A. cerana* colonies. The familiarised *A. dorsata* workers were not only accepted inside the colonies, but *A. cerana* guard bees also allowed them to enter the hives through the entrances. It is evident here, that relatively short familiarization predominated the acquired recognition cue over the heritable one.

The reciprocal experiment showed that *A. dorsata* did not damage unfamiliarised *A. cerana* brood introduced into their nests. Moreover, a strange phenomenon occurred. *A. dorsata* workers reconstructed the small *A. cerana* cappings into the large *A. dorsata* cappings without any damage to the brood. However, *A. dorsata* did not accept emerged *A. cerana* workers, despite the fact that they had been familiarised to the colonies by being in them as brood for several days. Thus, it is evident that *A. dorsata* uses heritable differences between both species as recognition cues in the recognition and rejection of adult *A. cerana* workers. Hence, the reciprocal reaction of the introduction of brood and young workers of one species into the nests of the other is just opposite.

Woyke *et al.* (2000 and 2001) showed that *A. mellifera* did not accept introduced unfamiliarised *A. dorsata* or *A. laboriosa* brood or adults. However, they accepted familiarised worker bees. In contrast, *A. dorsata* accepted unfamiliarised *A. mellifera* brood, but did not accept familiarised adult *A. mellifera* workers. Thus, the reaction of both cavity nesting bees, *A. cerana* and *A. mellifera* was similar for introduced *A. dorsata* brood or adults. The reaction of the free nesting *A. dorsata* for introduced brood or adults was similar for both cavity nesting species, *A. cerana* and *A. mellifera*. The acceptance and un-damage of introduced brood of other species into nests of *A. dorsata* is probably related to the hygienic behaviour of that bee. Cross fostering of brood and worker bees in cavity nesting *A. mellifera* and *A. cerana* colonies (Sakagami 1959, and Dhaliwal and Atwal 1970) showed that both species use acquired cues in recognition and acceptance of the other species. Hence, both

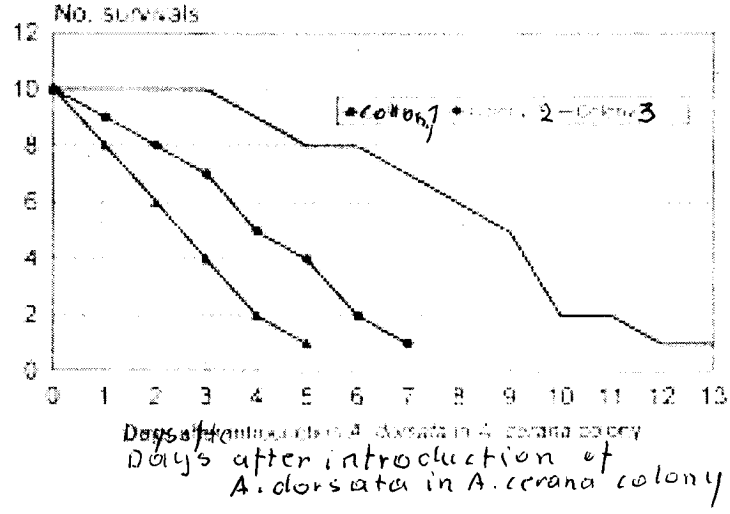
cavity nesting *A. cerana* and *A. mellifera* use recognition cues acquired by the introduced bees, in acceptance of the other species of both, the cavity nesting as well as the free nesting bees. Evidently, acquired recognition cues took precedence over heritable cues in both cavity-nesting bees.

Thus, familiarization of the introduced free living species makes it possible to keep them in the nests of the cavity nesting bee species. Hence, the knowledge of the recognition cues, permitted to elaborate a method to keep the free nesting *A. dorsata* worker bees in hives with *A. cerana* colonies. This allowed studying behaviour of the free nesting *A. dorsata* bees in changed living conditions.

CONCLUSION

A. cerana workers accepting *A. dorsata* bees use recognition cue acquired by *A. dorsata* in *A. cerana* colonies. *A. dorsata* workers, use inherited *A. cerana* recognition cue to eject workers of that species from their nests. It is possible to keep *A. dorsata* bees in *A. cerana* colonies. It is possible to investigate behaviour of *A. dorsata* bees in changed environmental conditions in *A. cerana* colonies.

Fig. 1. Survival of 10 *A. dorsata* workers introduced in small screen cages into *A. cerana* colonies.



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