Symposium 2

Coexistence of *Apis mellifera* and *Apis dorsata* Workers in the Same Colonies

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SUMMARY

Recognition of nestmate plays important role in evolution and social organisation of insects. We tested the recognition and acceptance question in extremely genetically different two *Apis* species. For this purpose, pieces of *A. dorsata* brood combs or young workers were introduced into *A. mellifera* colonies. In the reversal test, pieces of *A. mellifera* brood combs or caged young workers were introduced into *A. dorsata* nests. The results showed, that *A. mellifera* destroyed very quickly introduced *A. dorsata* brood. However, *A dorsata* workers ready to emerge begged for food and were fed by *A. mellifera* workers. Consequently emerging *A. dorsata* workers were accepted in *A. mellifera* colonies. Young *A. dorsata* workers emerged in an incubator and introduced into *A. mellifera* colonies were rejected. However, when such bees were at first kept in screen cages for one day in *A. mellifera* colonies they were accepted. Apparently, they became familiarised. *A. dorsata* workers survived in *A. mellifera* colonies for more than 50 days. They flew out and returned to *A. mellifera* hives. *A. dorsata* workers served also as guard bees in *A. mellifera* hives. Apparently, they learned the template cues of *A. mellifera* colony. The reciprocal test showed that *A. dorsata* did not destroy *A. mellifera* brood introduced into their nests. However, *A dorsata* did not accept young *A. mellifera* workers, despite they were familiarised as brood for 10 days. Hence, the reciprocal reaction of introduction of brood and workers of one species into nests of the other is just opposite. The results show, that *A mellifera* uses environmental cues to great extent in recognition and acceptance of the other species adults. *A. dorsata* uses genetic cues in recognition and rejection of the other species adults.

INTRODUCTION

The relationships between different species of honeybees are interesting from the behavioural point of view as well as from the phylogenetic one. According to Hamilton kinship theory (1964), the ability to recognise kin from non kin, or more related animals from less related played an important role in the evolution of social organisation in social insects. Breed (1983) demonstrated that honeybee workers attacked more frequently introduced unrelated than related workers. However, according to Downs and Ratnieks (1999) guard bees use nonheritable cues in recognition of conspecifics. Breed *at al.* (1995) showed that comb wax

plays important role in nestmate recognition in honeybees. It seems that both, heritable and environmental cues are used by *A. mellifera* in nestmate recognition.

We investigated the acceptance or rejection question in extremely genetic different *Apis* species. *A dorsata* brood and workers were introduced into *A. mellifera* colonies and vice versa. *A. dorsata* is a free nesting species and *A. mellifera* cavity nesting one.

MATERIAL AND METHODS

The investigations were conducted in Dabur Apicultural Center, Jugedi, Nepal from January 7 to May 3 1999 and from October 11. 1999 to January 15, 2000. Nine experiments were conducted:

1. Part of brood comb was cut from an *Apis dorsata* nest. Three pieces of about 8 x 12 cm containing young brood were selected. The brood consisted of eggs, larvae and sealed young pupae. Next sealed brood combs were removed from three *A. mellifera* colonies. Appropriate holes were cut in the centre of each comb. *A. dorsata* brood was inserted into the holes. The combs were returned to their colonies into the centre of the nests. Treatment of those combs by *A. mellifera* workers was checked 2.5 and 5 hours after introduction and one and two days later.
2. Similar experiment was repeated, except old sealed *A. dorsata* brood combs consisting of pupae with violet eyes and emerging workers were introduced into four *A. mellifera* colonies.
3. *A. dorsata* brood comb was put into incubator. Workers which emerged during the night were added in the morning to two *A. mellifera* colonies, 25 to one and 35 to the other. Workers, which emerged during the day, were added in the evening per 45 to two other *A. mellifera* colonies. Survival of those workers was recorded.
4. Similarly, per about 20 workers which emerged during the night were added in the morning to three mating nucei. The mating hives were of the Kirchhain type with 3 trapezoid combs 13 x 9 x 8 cm. The survival of the workers was noted.
5. Per 10 emerged *A. dorsata* workers were put into 6 small wire mesh cages 9 x 6 x 1 cm. They were located individually in the center of *A. mellifera* brood nests. Their survival was checked daily.
6. Ninety five *A. dorsata* workers which emerged in the incubator were put in a large wire mesh cage 20 x 20 x 2.5 cm. The cage was located in the morning in the center of *A. mellifera* nest. The number of survivals was counted the next day in the evening, when the cage entrance was opened. Number of *A. dorsata* workers in the colony was checked periodically.
7. Entrances of hives containing *A. dorsata* workers were observed periodically. The behaviour of guard bees toward both species was noticed.
8. Acceptance of *A. mellifera* brood and workers by *A. dorsata* was also investigated. For that purpose sealed brood combs were removed from *A. mellifera* colonies. Three pieces 10 x 10 cm were cut out. Two pieces contained old pupae and emerging workers. The third piece contained young pupae with pink eyes and few 4 day old larvae. Next, appropriate holes were cut out in brood area of three *A. dorsata* combs. The *A. mellifera* brood pieces were inserted into the free places. The three nests and combs were inspected daily.
9. Per 10 *A. mellifera* workers emerged in an incubator were put in 5 small wire mesh cages 9 x 6 x 1 cm. The cages with bees were introduced into 5 *A. dorsata* nests. Three repetitions were conducted within several days. The number of survivals was checked 1 and 2 days after introduction in the two first repetitions, and after 2 days in the third repetition.

RESULTS

Introduction of young brood

Examination of young *A. dorsata* brood introduced into *A. mellifera* colonies revealed that two and half hours after introduction, *A. dorsata* larvae 1 - 3 days old disappeared. However, eggs as well as larvae 4 days old were present there. Five hours after introduction, only some 4 days old larvae remained and part of them was damaged. However, the eggs were still present there. Wax was scratched from some caps of sealed

brood. Some cells were opened exposing white pupae. Other cells were empty (probably those containing larvae and prepupae). The next day after introduction, neither eggs nor larvae were found in the colonies. Wax was scratched from all brood sealings. About 1/3 of sealed brood cells was opened. Part of exposed pupae was eaten up. After two days all cells were opened, most of them were empty. Only 4, 12 and 20 damaged pupae were found in the three colonies. Thus, *A. mellifera* workers ate 1-3 day old *A. dorsata* larvae within 2.5 hours after introduction, eggs within 1 day and young pupae within 2 days.

Introduction of Old Brood

Examination of old *A. dorsata* brood in *A. mellifera* colonies revealed that two and half hours after introduction, wax was scratched out from some sealings. Some cocoons were punctured. Five hours after introduction some brood cells were opened. *A. mellifera* workers pulled *A. dorsata* pupae out of the cells. However, they did not pull out opened *A. dorsata* workers ready to emerge. Those *A. dorsata* workers were asking for food by antennae movements and stretching the tongue. *A. mellifera* workers fed them. Few of emerged *A. dorsata* workers were molested by *A. mellifera*. However, at the same time other *A. dorsata* workers were fed by *A. mellifera*. The next morning many old pupae were found before the entrances. Some were close to emerge. They were moving the legs and antennae. However, the wings were mostly not fully straightened. Apparently they were removed from the cells before being ready to emerge. *A. mellifera* workers pulling *A. dorsata* pupae out of the entrance tried to fly out with them. However, many times they were not able to lift them, and so left them before the entrances.

Inside the nests, all *A. dorsata* brood cells were opened the next day. Except 1 - 3 rows with emerging or ready to emerge *A. dorsata* workers, all other cells were empty. *A. mellifera* workers fed exposed *A. dorsata* workers ready to emerge from cells. Emerged *A. dorsata* workers moved freely on comb surface, only sporadically they were molested by *A. mellifera*. They were fed by *A. mellifera* workers. The next days, molestation of *A. dorsata* by *A. mellifera* was noticed very seldom, instead, feeding of *A. dorsata* by *A. mellifera* was observed.

When *A. mellifera* colonies were examined, many *A. dorsata* workers were found on the surface of the introduced piece of *A. dorsata* comb, during the first two days after emerging. Although, they left that piece during examination, they were found here again in the next check. In later days, congregation of *A. dorsata* workers on the piece of *A. dorsata* comb was not noticed.

A. dorsata workers survived in *A. mellifera* colonies for varying periods. The last *A. dorsata* workers were recorded in two colonies 10 and 12 days after introduction. However, Figure 1 shows that they survived much longer in colonies No 3 and No 13. The last *A. dorsata* workers were recorded there 69 and 45 days after introduction, respectively. The shorter survival in the two colonies was in great part due to drifting, because the *A. mellifera* colonies were only 30 - 50 cm apart from others.

Thus, *A. mellifera* workers remove *A. dorsata* pupae from brood cell within two days. However, they do not remove *A. dorsata* workers ready to emerge. Later both species coexist peacefully.

Introduction of Emerged Workers into Normal Colonies

After young *A. dorsata* workers (emerged in an incubator) were introduced into *A. mellifera* colonies, they were seen few minutes later being pulled out of the entrances. Out of 25 and 35 introduced in the morning into two colonies none remained in the colony till noon or till evening. Out of 45 introduced in the evening none was found in the morning in the third colony. However, out of 45 introduced to the forth colony 10 were detected the next morning. They were recovered also during inspections on three consecutive days. How long they survived was not recorded. Thus, *A. mellifera* mostly does not accept just emerged *A. dorsata* workers, and starts to remove them immediately. However, the few which survive the first day, probably may coexist in the colony for some time.

Introduction of Emerged Workers into Mating Nuclei

When emerged *A. dorsata* workers were introduced in the morning into small mating nuclei, some of them were molested by *A. mellifera* workers. However, they were not removed immediately, like it happened in normal colonies. Out of 21, 24 and 18 introduced workers 14, 16 and 12 were recovered in the nuclei the next morning respectively. Thus, 67% of introduced bees survived in all three nuclei. In the first nucleus, no any *A. dorsata* worker was found after two days. In the second, 8 and 1 were found after two and three days

respectively. In the third colony 12, 11, 6, 4, 2, and 1 workers were found 5, 9, 16, 17, 18 and 20 days after introduction, respectively. There was observed that great part of workers from the two first nuclei was lost due to drifting. Both nuclei were located very close (15 - 20 cm) to other mating hives. Thus, young *A. dorsata* workers survive in higher percentage in small colonies than in normal ones.

Introduction of Emerged Workers in Small Cages

Out of 10 young *A. dorsata* workers introduced into *A. mellifera* colonies in small cages, very different number survived different period of times. In 6 repetitions, the last alive bee was recorded 0, 2, 4, 14, 24 and 70 days after introduction. Distinct differences between colonies were found. In colony No 3, all 10 workers were still alive 27 days after introduction, and the last alive was noted 70 days after introduction. In colony No 7 the last alive bee was detected in two repetitions 0 and 4 days after introduction. Distinct differences in care of caged workers were found between honey flow in January and dearth period in the end of April. The last bee alive in the two periods was found in colony No 7 - 4 and 0 and in No 8, - 21 and 2 days after introduction, respectively.

Introduction of Emerged Workers in Large Cages

Out of 95 *A. dorsata* workers introduced in the morning into *A. mellifera* colony in large cage, 79 were alive the second evening (after 32 hours). After they were released, 73 were recovered the next morning and 68 the second one. Figure 1 (No 3. Apr) shows that 46 and 34 were found in the colony after 2 and 4 weeks respectively. The last one was recorded 58 days after introduction. Thus, this is the most effective method of introduction of *A. dorsata* workers into *A. mellifera* colonies.

Behaviour of Bees on Hive Entrances

A. dorsata workers were flying out and returning to *A. mellifera* hives. *A. mellifera* guard bees mostly allowed *A. dorsata* workers to enter the hives without any difficulties. Very rarely returning *A. dorsata* workers were molested for a short time by *A. mellifera* workers before entering the hives. Very interesting is to note, that some *A. dorsata* workers also served as guard bees. This activity was observed in bees older than two weeks. *A. dorsata* guard bees were active in the entrance or on the landing board. In the last case they located themselves on the upper surface or on the front edge of the board. On the front edge they sit head upwards. *A. dorsata* guard bees approached landing *A. mellifera* bees and checked them with antennae. It is evident that *A. dorsata* workers serving as guards learned the template cues of *A. mellifera* colony.

A. mellifera Brood in A. dorsata Nests

At first those two *A. dorsata* nests were examined to which *A. mellifera* brood ready to emerged was introduced. In the first colony 3/4 and in the second 1/4 of *A. mellifera* cells were empty the next day. No any damage of sealings of the remaining *A. mellifera* cells was noticed. Examination of the nests did not revealed any *A. mellifera* workers. However, *A. mellifera* workers were found on ground beneath the nests. The workers were alive and some were crawling. It looked like they were not stung, but expelled from the nests. The next day, almost all the cells were empty. We observed some emerging *A. mellifera* workers and opened some cells with workers ready to emerge. *A. mellifera* workers which emerged in our presence, were at first walking freely on the comb surface without any disturbance. However, at least they were molested by *A. dorsata* workers. As a result both or only *A. mellifera* worker felled down from the combs.

Examination of young *A. mellifera* brood introduced into the third *A. dorsata* colony, did not revealed any damage to the cappings. It is not clear whether the 4 day old larvae were sealed or removed. Five days after introduction no any damage to the sealings was noticed. Nine days after introduction some cells were already empty. However, sealings on the remaining were not damaged. We watched some emerging *A. mellifera* workers. The result was the same as described above.

Thus, contrary to *A. mellifera*, *A. dorsata* does not damage sealed brood of the other species. However, *A. dorsata* does not accept emerging bees of the other species, even when the brood was in the nest for 10 days.

Caged *A. mellifera* Workers in *A. dorsata* Nests

Survival of caged *A. mellifera* workers in 5 *A. dorsata* colonies was checked the next day only in the two first repetitions. Out of the total of 10 checks, all 10 bees were found alive 4 times, 8 bees - once, 6 - once, 2 - once and 0 - 3 times. None bee was found alive the second day after introduction in all tree repetitions. Thus, contrary to *A. mellifera*, the *A. dorsata* workers do not take care of caged bees of the other species.

DISCUSSION AND CONCLUSIONS

All results presented above show, that *A. mellifera* in normal colonies does not accept neither unfamiliar brood nor young *A. dorsata* workers. Begging of food released feeding response, and this probably resulted in familiarisation and subsequent acceptance of *A. dorsata* workers in *A. mellifera* colonies. *A. dorsata* workers kept in screen cages for one day in *A mellifera* colonies became familiarised and consequently were accepted. They survived in A. mellifera colonies for more than 50 days. It is interesting to note, that *A. dorsata* workers learned in *A. mellifera* colonies the template cues of the colony and used it as a guard bee to check entering *A. mellifera* foragers.

The reciprocal reaction showed, that *A. dorsata* did not damage unfamiliarised *A. mellifera* brood introduced into their nests. However, *A. dorsata* did not accept young *A. mellifera* workers despite they were familiarised to the colonies being in them as brood for 10 days.

Hence, the reciprocal reaction of introduction of brood and workers of one species into nests of the other is just opposite. The results show that even when extremely genetic different species are involved, *A mellifera* uses environmental cues to great extent in recognition and acceptance of workers of the other species. However, *A. dorsata* uses genetic cues in recognition and rejection of adults of the other species.

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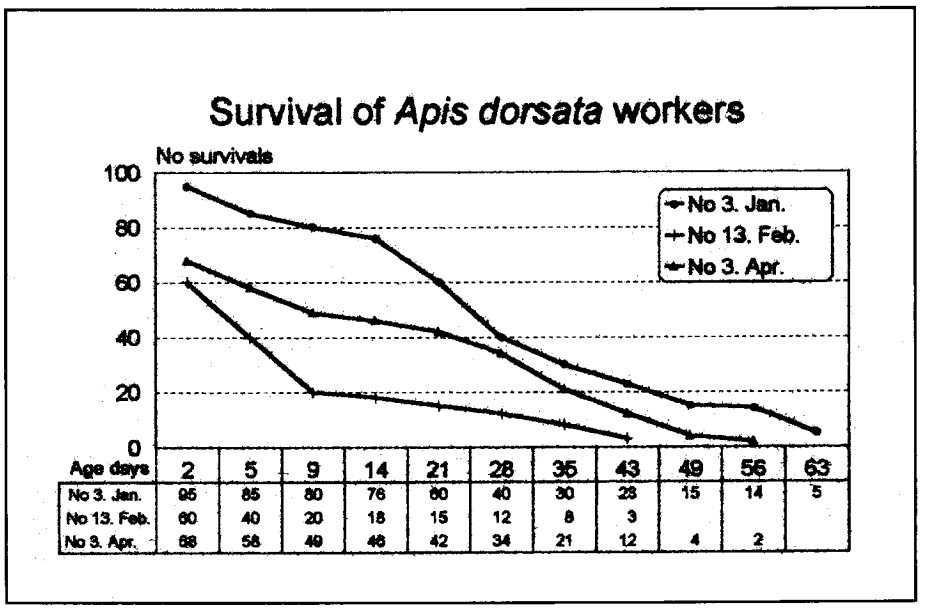
Table 1 Condition of *Apis dorsata* brood in determined time (hours) after introduction into *Apis mellifera* colonies. Averages from 3 colonies with young brood and 4 with old one

Brood	No cells	2.5 h	5 h	24 h	48 h
		Young	brood		
Eggs	80	present	present	all removed	-
1-3 d. larvae	80	80% removed	all removed	-	-
4 d. larvae	60	present	50% removed	all removed	-
Sealed	220	10% scratched	25% scratched	all scratched	15% opened
			5% opened	30% opened	rest empty
		Old	brood		
Sealed	440	40% scratched	40% opened with	15% emerging	all empty
		15% opened with	emerging workers	10% opened pupae	
		emerging workers	10% emerged	rest empty	

The removed young brood, as far as we have seen means brood eaten by worker bees

Figure 1 Survival of *Apis dorsata* workers in *Apis mellifera* colonies. In January and February brood combs with emerging *A. dorsata* workers was introduced into *A. mellifera* colonies. In April *A. dorsata* workers which emerged in incubator were introduced into *A. mellifera* colonies in large wire mesh cage.



Age days	2	5	9	14	21	28	35	43	49	56	63
No 3. Jan.	95	85	80	76	60	40	30	23	15	14	5
No 13. Feb.	60	40	20	18	15	12	8	3			
No 3. Apr.	68	58	49	46	42	34	21	12	4	2	

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