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GENETIC VERSUS ENVIRONMENTAL CUES USED IN THE RECOGNITION AND ACCEPTANCE OF TWO HONEYBEE SPECIES: *APIS MELLIFERA* AND *APIS DORSATA* IN THE SAME COLONY

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Summary

The recognition of nestmates plays an important role in the evolution and social organisation of insects. We tested the recognition and acceptance question in two extremely genetically different populations of two *Apis* species. For this purpose, pieces of *A. dorsata* brood combs, or young workers, were introduced into *A. mellifera* colonies. In a reverse test, pieces of *A. mellifera* brood combs, or caged young workers, were introduced into *A. dorsata* nests. The results showed that *A. mellifera* very quickly destroyed the 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. The familiarisation of *A. dorsata* brood for 1 or 2 days increased the number of emerged and accepted adults by 9 - 11 times. The young *A. dorsata* workers which emerged in an incubator and were introduced into *A. mellifera* colonies were rejected. However, when such bees were at first kept in wire mesh 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. They were permitted by *A. mellifera* guard bees to enter the hives. *A. dorsata* workers served also as guard bees in *A. mellifera* hives. Apparently, they learned the template cues of the *A. mellifera* colony. The reciprocal test showed that *A. dorsata* did not destroy the *A. mellifera* brood introduced into their nests. However, *A. dorsata* did not accept the young *A. mellifera* workers, despite the fact that they were familiarised as brood for 10 days. Hence, the reciprocal reaction of the introduction of brood and workers of one species into the nests of the other is just the opposite. The results show that *A. mellifera* uses environmental cues to a great extent in the recognition and acceptance of the other adult species. *A. dorsata* uses genetic cues in the recognition and rejection of the other adult species.

Keywords: *Apis dorsata*, *Apis mellifera*, genetic cue, environmental cue, crossfostering, acceptance, recognition.

INTRODUCTION

The relationships between different species of honey bees are interesting from the behavioural point of view as well as from the phylogenetic one.

According to the 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 a social organisation in social insects. Breed (1983) demonstrated that *Apis mellifera* attacked the introduced unrelated workers more frequently than the related ones, which had been kept previously in the same incubator conditions. Getz and Smith (1983) showed that honey bee workers attacked introduced half-sisters more frequently than full sisters, which had been kept previously in the same conditions. Moritz and Hillesheime (1990) determined that worker bees fed closely related bees more frequently than unrelated ones. All the authors concluded that honey bees used genetic cues in the recognition of *A. mellifera* workers. However, according to Downs and Ratnieks (1991) guard bees use non-heritable cues in recognition of conspecifics. Breed et al. (1995) showed that comb wax plays an important role in nestmates recognition in honey bees. It seems that both heritable and environmental cues are used by *A. mellifera* in nestmate recognition.

Sakagami (1959) and Dhaliwal and Atwal (1970) investigated heterospecific relations between two cavity-nesting species. *A. mellifera* and *A. cerana*. They introduced brood and workers of one species into the nests of the other. Pothichot et al. (1993) conducted interspecific queen rearing of *A. cerana* and *A. mellifera*, and Koeniger et al. (1996) of *A. cerana* and *A. koschevnikovi*. Koeniger et al. (1996) cross-fostered drones of *Apis cerana* and of *Apis koschevnikovi*. All these investigations concerned cavity-nesting bees.

We investigated the acceptance or rejection question in extremely genetically different species, the multicomb cavity-nesting *A. mellifera* and the monocomb free-living *A. dorsata* (Ruttner 1988)

MATERIALS AND METHODS

The investigations were conducted at the Dabur Apiculture Centre, Jugedi, Chitwan, Nepal from January to May 1999 and from October 1999 to June 2000. Ten experiments were conducted:

A. In order to investigate whether *A. mellifera* accepts *A. dorsata* brood, three experiments were conducted:

1. Introduction of young brood. Part of a brood comb was cut from an *Apis dorsata* nest. Three pieces measuring 5 x 11 cm containing 440 cells of young brood were selected. The brood consisted of half eggs and larvae and half of sealed young pupae. Next, sealed brood combs were removed from three *A. mellifera* 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. The treatment of those combs by *A. mellifera* workers was checked 2.5 and 5 hours after introduction and 1 and 2 days later.

2. Introduction of an old brood. A similar experiment was repeated, but this time using old sealed *A. dorsata* brood combs consisting of pupae with violet eyes and emerging workers were introduced into four *A. mellifera* colonies.

3. Familiarisation of *A. dorsata* brood in *A. mellifera* colonies. Two pieces of *A. dorsata* old brood (8 x 10 cm) were introduced into *A. mellifera* colonies. One piece was unprotected and the other was covered with wire mesh. In 3 colonies, the screens were removed after 24 hours and in 2 after 48 hours. The numbers of scratched cappings, removed pupae and emerged workers in unprotected and protected combs were compared.

B. In order to investigate whether *A. mellifera* accepts adult *A. dorsata* workers, the following five experiments were conducted:

4. Introduction of emerged *A. dorsata* workers into normal *A. mellifera* colonies. *A. dorsata* brood comb was put into an 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, 45 to each of two other *A. mellifera* colonies. The survival rates of those workers were recorded.

5. Introduction of emerged *A. dorsata* workers into *A. mellifera* mating nuclei. Similarly, about 20 workers, which emerged during the night, were added in the morning to each of three mating nuclei. The mating hives were of the Kirchhain type with 3 trapezoid combs 13 x 9 x 8 cm. The survival rates of the workers were noted.

6. Introduction of emerged *A. dorsata* workers in small cages into *A. mellifera* colonies. Ten emerged *A. dorsata* workers were put into each of 6 small wire mesh cages 9 x 6 x 1 cm. They were located individually in the centre of six *A. mellifera* brood nests. Their survival rate was checked daily.

7. Introduction of emerged *A. dorsata* workers in large cages into *A. mellifera* colonies. Ninety five *A. dorsata* workers which emerged in the incubator were put into a large wire mesh cage 20 x 20 x 2.5 cm. The cage was located in the morning in the centre of *A. mellifera* nest. The number of survivors was counted the next day in the evening, when the cage entrance was opened. The number of surviving *A. dorsata* workers in the colony was checked periodically.

8. Behaviour of *A. dorsata* workers at hive entrances of *A. mellifera* colonies. The entrances of *A. mellifera* hives containing *A. dorsata* workers were observed periodically. The behaviour of guard bees towards both species was noted.

C. In order to investigate whether *A. dorsata* accepts *A. mellifera* brood or adult workers, the following two experiments were conducted:

9. Introduction of *A. mellifera* brood into *A. dorsata* nests. 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 a few 4-day old larvae. Next, appropriate holes were cut out in the brood area of three *A. dorsata* combs. The *A. mellifera* brood pieces were inserted into the three places. The three nests and combs were inspected daily.

10. Caged *A. mellifera* workers in *A. dorsata* nests. Per 10 *A. mellifera* workers which had emerged in an incubator were placed 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 survivors was checked 1 and 2 days after introduction in the two first repetitions, and after 2 days in the third repetition.

RESULTS

1. Introduction of young *A. dorsata* brood into *A. mellifera* colonies

Table 1

Condition of *A. dorsata* brood in determined time (hours) after introduction into *A. mellifera* colonies. Averages from 3 colonies with young brood and 4 with old brood - Stan czerwiu *Apis dorsata* w określonych godzinach po umieszczeniu w gniazdach A *mellifera*. Średnie z 3 rodzin z młodym czerwiem i z 4 ze starszym

Brood age Wiek czerwiu	Hours after introduction - Godzin po umieszczeniu				
	No cells Szt. kom.	2.5	5	24	48
A.	Young brood - Czerw młody				
Eggs Jaja	80	present obecne	present obecne	all eaten zjedzone	- -
1-3 d. larvae 1-3 d. larwy	80	80% eaten 80% zjedzone	all eaten zjedzone	- -	- -
4 d. larvae 4 d. larwy	60	present obecne	50% eaten 50% zjedzone	all removed zjedzone	- -
Sealed Kryty	220	10% scratched 10% zeskrobane	25% scratched 25% zeskrobane 5% opened 5% otwarte	all scratched zeskrobane 30% opened 30% otwarte	15% opened rest empty 15% otwarte reszta puste
B.	Old brood - Czerw starszy				
Sealed Kryty	440	40% scratched 40% zeskrobane 15% opened, with emerging workers 15% otwarte z wygryzającymi się robotnicami	40% opened with emerging workers 40% otwarte z wygryzającymi 10% emerged 10% wygryzione	15% emerging 15% wygryz. 10% opened pupae, rest empty 10% otwarte reszta puste	all cells empty wszystkie komórki puste

Table 1 (A.) shows that 2.5 hours after the introduction of the young *A. dorsata* brood into *A. mellifera* colonies, 80% of 1-3 day-old larvae had disappeared. As far as we have seen the larvae were eaten. However, eggs, as well as larvae 4 day-old, were still present there. The sealings on capped brood were scratched in 10% of cases. Five hours after the introductions only 50% of 4 day-old larvae remained and some of them were damaged. However, the eggs were still present there. Wax was scratched from 25% of the caps of the sealed brood. About 5% of 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. The wax was scratched from all the brood sealings. About 1/3rd of sealed brood cells was opened. Part of the exposed pupae was eaten up. After two days all cells were opened, most of them were empty. Only about 15% of the damaged pupae remained in the three colonies. Thus, *A. mellifera* workers ate most of the 1-3 day-old *A. dorsata* larvae within 2.5 hours after introduction, eggs within 1 day and young pupae within 2 days.

2. Introduction of old *A. dorsata* brood into *A. mellifera* colonies

Table 1 (B.) shows that 2.5 hours after the introduction of old *A. dorsata* brood into *A. mellifera* colonies, the wax was scratched out from about 40% of sealings. About 15% of cells with emerging workers were opened. Five hours after the introduction 40% of 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 their tongues. *A. mellifera* workers fed them. *A. dorsata* workers had already emerged from 10% of cells. Some of them were molested by *A. mellifera*. The *A. mellifera* workers pulled the lags or wing of *A. dorsata* workers. They approached *A. dorsata* workers and made movements which looked as if they were biting the body surface. However, they did not sting *A. dorsata* workers. At the same time, other *A. mellifera* fed other emerged *A. dorsata* workers.

The next morning many old pupae were found in front of the entrances. Some were close to emerging. They were moving legs and antennae. However, the wings were mostly not fully straightened. Apparently, they had been 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, often they were not able to lift them, and so left them in front of the entrances.

Inside the nests, all *A. dorsata* brood cells were opened the next day. Only 15% of cells contained emerging or ready-to-emerge *A. dorsata* workers. In 10% of cells, open pupae remained. All the 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 being molested by *A. mellifera* workers. A. mellifera workers fed them. The next days, the molestation of *A. dorsata* by *A. mellifera* was noticed only rarely. Instead, the feeding of *A. dorsata* by *A. mellifera* was observed.

Table 2

Treatment of *A. dorsata* brood introduced into *A. mellifera* colonies. The brood was unprotected (NO) or protected with wire mesh for 24 or 48 h (YES). The number of scratched cappings (SC), removed pupae (RP) and emerged *A. dorsata* workers (EW) - Traktowanie czerwiu *A. dorsata* umieszczonego w rodzinach *A. mellifera*. Czerw albo nie był chroniony (Nie) albo był ochraniany siatką przez 24 lub 48 godz. (Tak). Liczba zeskrobanych zasklepów (SC), usuniętych poczwarek (RP) i wygryzionych robotnic *A. dorsata* (EW)

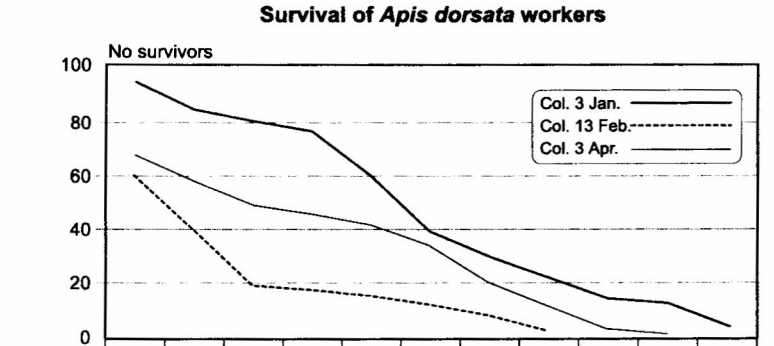
Colony No Nr rodziny	Protected Chroniony hours godz.	Hours after introduction into *A. mellifera* colonies Godziny po umieszczeniu w rodzinach *A. mellifera*								
		2.5		5		24		48		72
		SC	RP	SC	RP	SC	RP	SC	RP	EW
31	No Nie	79	15	135	38	344	217	365	356	42
	Yes Tak 24	32	2	55	22	63	42	69	65	295
17	No Nie	93	25	150	74	281	156	360	265	50
	Yes Tak 24	27	5	47	32	85	68	97	74	278
38	No Nie	82	31	179	74	342	211	358	217	38
	Yes Tak 24	15	3	18	11	21	13	23	21	543
Mean Średnia	No Nie	85b	24b	155b	62b	322b	195b	361b	279b	43a
	Yes Tak 24	25a	3a	40a	22a	56a	41a	63a	53a	372b
11	No Nie	98	47	220	88	462	330	512	352	45
	Yes Tak 48	15	3	22	11	24	15	28	22	503
17	No Nie	106	36	217	87	403	266	431	277	44
	Yes Tak48	14	2	21	10	22	12	24	23	458
Mean Średnia	No Nie	102b	42b	219b	88b	433b	298b	472b	315b	45a
	Yes Tak48	15a	3a	22a	11a	23a	14a	26a	23a	481b

Different letters behind the means indicate statistically significant differences
Różne litery za średnimi oznaczają statystycznie istotne różnice

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 the examination, they were found there again in the next check. After few days, the 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 their introduction. However, fig. 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 their introduction, respectively. The shorter survival in the two first colonies might have been due to drifting, because the *A. mellifera* colonies were only 30 - 50 cm apart from the others.



Age days	2	5	9	14	21	28	35	43	49	56	63
Col. 3 Jan.	95	85	80	76	60	40	30	23	15	14	5
Col. 13 Feb.	60	40	20	18	15	12	8	3			
Col. 3 Apr.	68	58	49	46	42	34	21	12	4	2	

Fig. 1 Survival of *A. dorsata* workers in *A. mellifera* colonies. In January and February, brood combs with emerging *A. dorsata* workers was introduced into *A. mellifera* colonies Nos 3 and 13. In April, *A. dorsata* workers which emerged in incubator were introduced into *A. mellifera* colony No. 3 in large wire mesh cage.
Przeżywalność robotnic *A. dorsata* w rodzinach *A. mellifera*. W styczniu i lutym poddano wygryzający się czerw *A. dorsata* do rodzin *A. mellifera* Nr 3 i 13. W kwietniu, robotnice *A. dorsata* wygryzione w cieplarce poddano do rodziny Nr 3 w dużej osiatkowanej klatce. No survivors - liczba żywych, Age days – age of survived *A. dorsata* workers, - wiek w dniach przeżywających robotnic *A. dorsata*.

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

3. Familiarisation of *A. dorsata* brood in *A. mellifera* colonies

Table 2 shows that fewer cappings on *A. dorsata* brood were scratched in pieces which had previously been protected with wire mesh for 24 hours than in unprotected ones. The results of all four checks (2.5 - 48 hours) were highly significantly lower for protected than for unprotected pieces of brood. Highly significantly, fewer pupae were removed from pieces previously protected for 24 hours than from unprotected ones. On average, 8.7 times more adult *A. dorsata* workers emerged from previously protected than from unprotected combs.

Equally significantly, in combs protected for 48 hours, fewer cappings were scratched and fewer pupae were removed from pieces previously

protected than from unprotected. On average, 10.7 times more adult *A. dorsata* workers emerged from combs protected for 48 hours than from unprotected ones.

The statistical comparison of results concerning brood combs protected for different time showed that significantly fewer cappings were scratched and fewer pupae were removed in combs protected previously for 48 than for 24 hours. Significantly more *A. dorsata* adults emerged from combs protected previously for 48 than 24 hours.

Thus the familiarisation of *A. dorsata* brood in *A. mellifera* colonies for 24 hours decreased the number of damaged brood and increased the number of emerged adults. Familiarisation for 48 hours decreased still further the number of damaged brood and increased the number of emerged adults.

4. Introduction of emerged *A. dorsata* workers into normal *A. mellifera* colonies

After young *A. dorsata* workers which emerged in an incubator were introduced into *A. mellifera* colonies, they were seen being pulled out of the entrances a few minutes later. Out of 25 and 35 introduced in the morning into two colonies, none remained in the colony until noon or until evening. Out of 45 introduced in the evening, none was found the next morning in the third colony. However, out of 45 introduced to the fourth 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 newly-emerged *A. dorsata* workers, and starts to remove them immediately. However, the few which do survive the first day may probably survive in the colony for some time.

5. Introduction of emerged *A. dorsata* workers into *A. mellifera* mating nuclei

When emerged *A. dorsata* workers were introduced in the morning into small mating nuclei, *A. mellifera* workers molested some of them. However, they were not removed immediately, as happened in normal colonies. Out of 21, 24 and 18 introduced workers, 14, 16 and 12 respectively were recovered in the nuclei the next morning. Thus, 67% of the introduced bees survived in all three nuclei. In the first nucleus, no *A. dorsata* workers were 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 respectively after their introduction. It was observed that a great part of the 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.

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

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

7. Introduction of emerged *A. dorsata* workers in large cages into *A. mellifera* colonies

Out of 95 *A. dorsata* workers introduced in the morning into an *A. mellifera* colony in a large cage, 79 were alive on the second evening (after 32 hours). Following their release, 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 the introduction. Thus, this is the most effective method of introducing *A. dorsata* workers into *A. mellifera* colonies.

8. Behaviour of *A. dorsata* workers at the hive entrances of *A. mellifera* colonies

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

9. Introduction of *A. mellifera* brood into *A. dorsata* nests

At first, those two *A. dorsata* nests were examined into which *A. mellifera* brood ready to emerge had been introduced. In the first colony 3/4 and in the second 1/4 of *A. mellifera* cells were empty the next day. No damage to the sealings of the remaining *A. mellifera* cells was noticed. An examination of the

nests did not reveal any *A. mellifera* workers. However, *A. mellifera* workers were found on the ground beneath the nests. The workers were alive and some were crawling. It looked as if they had not been stung, but had been 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 able to walk freely on the comb surface without any disturbance. However, later a few *A. dorsata* workers molested them. As a result both or only *A. mellifera* workers fell down from the combs.

An examination of the young *A. mellifera* brood introduced into the third *A. dorsata* colony did not reveal any damage to the cappings. It is not clear whether the 4 day-old larvae had been sealed or removed. Five days after brood introduction no damage to the sealings was noticed. Nine days after introduction some cells were already empty. However, sealings on the remainder 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 a sealed brood of the other species. However, *A. dorsata* does not accept emerging bees of the other species, even when the brood has been in the nest for 10 days.

10. Caged *A. mellifera* workers in *A. dorsata* nests

The survival of caged *A. mellifera* workers in 5 *A. dorsata* colonies was checked the next day only in the two first repetitions. Out of a total of 10 checks, all 10 bees were found alive 4 times, 8 bees - once, 6 - once, 2 - once and 0 - 3 times. No bees were found alive the second day after their introduction in all three repetitions. Thus, contrary to *A. mellifera*, *A. dorsata* workers do not take care of caged bees of the other species.

DISCUSSION AND CONCLUSIONS

All the results presented above show that *A. mellifera* in normal colonies does not accept either unfamiliar *A. dorsata* brood or young *A. dorsata* workers. Here, both genetic and environmental cues could result in the rejection of the other species. However, begging for food invoked a feeding response and this probably resulted in familiarisation and subsequent acceptance of *A. dorsata* workers in *A. mellifera* colonies. Familiarisation of *A. dorsata* brood for 1 or 2 days decreased significantly the number of removed pupae, and increased the number of emerged and accepted adults by a factor of 9 - 11 times. The significance of an environmental cue is evident here. In small *A. mellifera* colonies, some *A. dorsata* workers were accepted despite the fact that they were not familiarised. *A. dorsata* workers kept in wire mesh cages for one day in A *mellifera* colonies became familiarised and consequently were accepted by *A. mellifera*. Thus, a relatively short familiarisation enhanced the environmental cues over the genetic ones. The

familiarised *A. dorsata* workers were not only accepted inside the colonies, but the guard bees also allowed them to enter the hives through the entrances. They survived in *A. mellifera* colonies for more than 50 days. It is interesting to note that *A. dorsata* workers in *A. mellifera* colonies learned the template cues of the colony and used them as a guard bee would to check entering *A. mellifera* foragers.

Downs and Ratnieks (1991) demonstrated the precedence of environmental cues over inherited ones in recognition by honey bees. They explained that the inherited cues in recognition demonstrated by Breed (1983), Getz and Smith (1983) and Moritz and Hillesheim (1990) were used by bees in laboratory conditions when environmental cues were artificially minimised. Our results demonstrated that even with the extreme genetic diversity of two *Apis* species, *A. mellifera* preferred environmental cues over genetic ones in recognition and acceptance of the other species. Although, all *A. dorsata* workers in *A. mellifera* colonies were of the same age, only a few served as guards. It may be that only some *A. dorsata* individuals of a rare genotype undertake this task, in the same way as was suggested for *A. mellifera* by Robinson and Page (1988)

The reciprocal reaction showed that *A. dorsata* does not damage unfamiliarised *A. mellifera* brood introduced into their nests. However, the congregation of *A. dorsata* workers on pieces of *A. dorsata* combs in *A. mellifera* colonies indicates that *A. dorsata* workers are able to distinguish between *A. mellifera* and *A. dorsata* combs. Nonetheless, the workers in *A. dorsata* colonies do not use that cue for opening cappings on sealed cells that contained brood of the other species.

However, *A. dorsata* did not accept young *A. mellifera* workers, despite the fact that they had been familiarised to the colonies by being in them as brood for 10 days. *A. dorsata* also did not take care of young *A. mellifera* workers introduced into the nests in small cages. Those *A. mellifera* workers, which survived the first day in cages in *A. dorsata* colonies, could become familiarised. Nevertheless, *A. dorsata* did not take care of them. Thus it is evident that *A. dorsata* uses genetic cues in the recognition and rejection of *A. mellifera* workers.

Hence, the reciprocal reaction of the introduction of brood and workers of one species into the nests of the other is just the opposite. *A. mellifera* does not accept unfamiliarised brood; however it accepts familiarised workers of *A. dorsata*. *A. dorsata*, accepts unfamiliarised *A. mellifera* brood, but not adults.

Dhalival and Atwal (1970) and Sakagami (1959) showed that *A. mellifera* and *A. cerana* did not accept unsealed brood of the other species but did, however, accept the sealed one. Thus, the reaction for unsealed brood of the other species was the same as in *A. dorsata* and *A. mellifera*. *A. dorsata* also accepted the sealed brood of the other species, like the two above.

However, *A. mellifera* did not accept the sealed brood of *A. dorsata*, contrary to the acceptance of the sealed *A. cerana* brood. Thus the cavity-nesting *A. mellifera* species accepted the sealed brood of the other cavity-nesting species, while it rejected the brood of the free-living species. According to Sakagami (1959), *A. mellifera* did not accept emerging *A. cerana* workers, just as *A. dorsata* did not accept *A. mellifera* in our investigations. According to Dhaliwal and Atwal (1970), *A. mellifera* accepted emerging *A. cerana* workers, just as *A. mellifera* accepted emerging *A. dorsata* workers in our investigations. Heterospecific queen rearing of *A. mellifera* and *A. cerana* resulted in the rejection of larvae of one species in the nests of the other (Pothichot et al. (1993). *Apis cerana* colonies accepted *Apis koschevnikovi* queens but no grafted queen larvae. *Apis koschevnikovi* colonies accepted *Apis cerana* grafted larvae but no *Apis cerana* queens (Koeniger et al. 1996). According to our observations *A. dorsata* larvae introduced into *A. mellifera* nests, either in comb cells or grafted in queen cups, were treated very differently (Woyke *et al.* 2001 in preparation). The cross-fostered drone brood of *A. cerana* and *A. koschevnikovi* was accepted both ways (Koeniger et al. 1994).

All the results show that the reciprocal reaction to the introduction of one species of bees into the nests of the other is different in different species.

The results show that even when more phylogenetic distant species are involved, *A. mellifera* seems to depend on environmental cues to a great extent in the recognition and acceptance of workers of the other species. However, *A. dorsata* uses genetic cues in the recognition and rejection of adults of the other species.

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GENETYCZNE CZY ŚRODOWISKOWE SYGNAŁY UŻYWANE W ROZPOZNAWANIU I AKCEPTACJI DWU GATUNKÓW PSZCZÓŁ: *APIS MELLIFERA* I *APIS DORSATA* W TEJ SAMEJ RODZINIE

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Streszczenie

Rozpoznawanie współtowarzyszy gniazda odgrywa ważną rolę w ewolucji i organizacji społecznej owadów. Badaliśmy zagadnienie rozpoznawania i akceptacji dwu genetycznie skrajnych populacji pszczół, *Apis mellifera* i *Apis dorsata*.

Badania prowadzono w Centrum pszczelarskim Dabur w Jugedi, w rejonie Chitwan w Nepalu w okresie od stycznia do maja 1999 r. i od października do czerwca 2000 r. Przeprowadzono 10 doświadczeń. W celu zbadania akceptacji czerwiu *A. dorsata* przez *A. mellifera* umieszczano w gnieździe tej ostatniej kawałki czerwiu; 1/ młodego, 2/ starszego oraz 3/ starszego zabezpieczonego siatką przed dostępem robotnic przez 1 lub 2 dni. W celu zbadania akceptacji dorosłych robotnic *A. dorsata* przez *A. mellifera*, czerw tej pierwszej umieszczano w cieplarce. Wygryzione robotnice poddawano; 4/ bezpośrednio do

normalnych rodzin *A. mellifera*, 5/ do ulików weselnych, 6/ umieszczano w gnieździe po 10 w małych drucianych klateczkach nie uwalniając ich oraz 7/ umieszczano najpierw w dużych klateczkach (20 x 20 x 2,5 cm) w gnieździe celem przejścia zapachem, po czym uwalniano je, 8/ obserwowano zachowanie robotnic *A. dorsata* na wylotku ula z *A. mellifera*. W celu zbadania akceptacji czerwiu lub robotnic *A. mellifera* przez *A. dorsata* umieszczano; 9/ kawałki czerwiu tej pierwszej w gniazdach drugiej, oraz 10/ umieszczano po 10 młodych robotnic *A. mellifera* w małych klateczkach w gniazdach *A. dorsata*.

Wyniki badań (tab 1) wykazały, że robotnice *A. mellifera* już po 2.5 godz. zjadły większość młodych larw *A. dorsata* i zaczęły niszczyć zasklep. Następnego dnia zjadały lub wynosiły poczwarki. Jednak robotnice *A. dorsata* gotowe do wygryzienia prosiły o pokarm i były karmione przez robotnice *A. mellifera*. Wygryzione zostały zaakceptowane w gnieździe *A. mellifera*. Robotnice *A. mellifera* zaakceptowały 9 - 11 razy więc robotnic *A. dorsata* z plasterka który przechodził zapachem przez 1 - 2 dni, niż ze świeżo poddanego (tab. 2). Młode robotnice *A. dorsata* poddane bezpośrednio do normalnych rodzin *A. mellifera* zostały wkrótce usunięte. Jednak niektóre poddane do małych rodzinek w ulikach weselnych zostały zaakceptowane. Robotnice *A. dorsata* przetrzymywane w małych klateczkach przeżyły w gniazdach *A. mellifera* od 0 do 70 dni. Gdy młode robotnice *A. dorsata* w dużych klateczkach przeszły zapachem *A. mellifera* to po uwolnieniu zostały zaakceptowane. Przeżyły one ponad 50 dni w rodzinach *A. mellifera* (fig. 1). Wylatywały i wracały do uli, a na wylotku wachlowały i pełniły rolę strażniczek.

Odwrotne badania wykazały, że robotnice *A. dorsata* nie niszczyły czerwiu *A. mellifera* wstawionego do ich gniazd. Usuwały jednak wygryzione robotnice, pomimo, że te jako czerw przechodziły zapachem *A. dorsata* nawet przez 10 dni. Robotnice *A. mellifera* w małych klateczkach w gniazdach *A. dorsata* padły w ciągi 1 - 2 dni.

Tak więc reakcja na czerw lub robotnice jednego gatunku poddane do gniazd drugiego jest odwrotna. *A. mellifera* niszczy poddany czerw *A. dorsata* lecz akceptuje czerw i dorosłe robotnice, które przeszły ich zapachem. *A. dorsata* nie niszczy czerwiu *A. mellifera*, lecz usuwa nawet te robotnice, które przeszły jej zapachem. Tak więc widać, że *A. mellifera* kieruje się w dużym stopniu sygnałami środowiskowymi w akceptacji drugiego gatunku. Natomiast *A. dorsata* kieruje się sygnałami genetycznymi w akceptacji dorosłych osobników *A. mellifera*.

Słowa kluczowe: *Apis dorsata*, *Apis mellifera*, sygnały genetyczne, sygnały środowiskowe, współistnienie, rozpoznawanie, akceptacja.