Coexistence and Behavior of Phylogenetically Distant Honey Bee *Apis laboriosa* in *Apis mellifera* Colony

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Abstract

The investigations were conducted in 1999/2000 in the Himalayas and in Jugedi, Chitwan, Nepal. *Apis laboriosa* brood comb was cut from one nest and was put into an incubator. The emerged *A. laboriosa* workers were introduced into *A. mellifera* colonies in different ways. The results show, that *A. mellifera* did not accept *A. laboriosa* workers introduced directly into the colonies. However, after familiarization in the colonies, in cages, for one day, *A. mellifera* accepted them. Already 2 days old *A. laboriosa* workers flew out of the hive. The workers retained the flight pattern characteristic of free living bees. However, young *A. laboriosa* workers in *A. mellifera* colonie did not fly within the short periodic mass flight activity characteristic of the open-air-nesting bees.

INTRODUCTION

Every beekeeper knows that honey bees recognize worker bees from other colonies. They attack any intruder bee trying to enter the colony. Also, foreign worker bees introduced into a colony are attacked and removed from the nest.

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

Sakagami (1959) and Dhaliwal and Atwal (1970) investigated heterospecific relations between two cavity nesting species, *A. mellifera* and *A. cerana*. However, Woyke et al. (2000 and 2001), investigated recognition and acceptance of two phylogenicaly distinct species; the cavity-nesting *A. mellifera* and *A. cerana* and the open-air-nesting *A. dorsata*.

In this study, the authors intend to test which type of recognition cues, the heritable or the environmental, acquired by the introduced species, is used by *A. mellifera* in acceptance or rejection of *A. laboriosa*. The cavity-nesting *A. mellifera* is phylogenicaly distant from the open-air-nesting *A. laboriosa*. Will the recognition cues acquired by the introduced species in the host colony of the other species override the large heritable differences between those species? Is it possible to keep the two very different bee species in one colony? This would enable us to study behavioural and other features of one bee species in changed environmental conditions in the colony of the other species.

MATERIALS AND METHODS

The investigations were conducted in Nepal in December 1999 and January 2000. *A. laboriosa* brood comb was collected on December 1999 from a colony at a rock cliff with 53 nests in the Himalayas in Chale

(lat. 28°09' N., long. 85°48' E., alt. 1500 m), in Sindhupalchok district at the Bhote Koshi river near Kodari at Tibetan border. Further investigations were conducted at the Dabour Apiculture Centre in Jugedi, Chitwan.

In order to investigate whether *A. mellifera* accepts adult *A. laboriosa* worker bees, pieces of *A. laboriosa* brood combs were put into an incubator at 34°C. After some workers emerged they were introduced into *A. mellifera* colonies in three different ways.

1. Thirty *A. laboriosa* workers each were added directly into two *A. mellifera* colonies.
2. Ten *A. laboriosa* workers each were put into each of 5 small wire mesh cages 9 cm x 6 cm x 1 cm. They were located individually in the centre of five *A. mellifera* brood nests.
3. 120 and 75 emerged *A. laboriosa* workers were put into two large wire mesh cages 20 x 20 x 2.5 cm. The cages were located in the centre of two *A. mellifera* nests. The cage entrance was opened the next day so that the workers could come out. The number of *A. laboriosa* workers surviving in all *A. mellifera* colonies was checked daily.

The entrances of *A. mellifera* hives containing *A. laboriosa* workers were observed daily. The number of flying *A. laboriosa* workers, as well as the time of their flights, was recorded. The behavior of guard bees towards both species was also noted.

All of the observations are presented on video.

RESULTS

Introduction of *A. laboriosa* worker bees directly into *A. mellifera* colonies.

After 30 young *A. laboriosa* workers were introduced directly into each of two *A. mellifera* colonies, they were seen a few minutes later being pulled out of the entrances of both hives. The next day, no *A. laboriosa* worker was found in both colonies.

Introduction of *A. laboriosa* workers in small cages

After five cages, each with 10 *A. laboriosa* workers which emerged in an incubator, were added into five *A. mellifera* colonies, all workers were found alive 4 days later (Fig. 1). After 10 days 2 to 10 workers were alive, and after 15 days 0 to 7 bees. The maximal survival time is not known, because the experiment had to be terminated. Thus, the cage protected unfamiliarized *A. laboriosa* workers from being ejected from *A. mellifera* colonies immediately after introduction. However, after *A. laboriosa* workers became familiarized, some of them were fed by *A. mellifera* worker bees for more than two weeks.

Introduction of *A. laboriosa* workers in large cages and releasing them

The day after *A. laboriosa* workers were released from the two large cages into two *A. mellifera* colonies, 106 were found in colony 3 and 70 in colony 4 (Fig. 2). Of the 100% workers found in the colonies on the first day after introduction, 80% and 61% were found 3 days later and 8% and 34%, 15 days later in the two colonies, respectively. No daily examinations were conducted after 16 days. However, 35 days after introduction, no *A. laboriosa* worker was found in colony 3, but five (7%) in colony 4. Thus, a short familiarization of *A. laboriosa* for 1 day in *A. mellifera* colonies sufficed for their acceptance by *A. mellifera* bees.

Flight activity of *A. laboriosa* in *A. mellifera* colony

Fig. 3 shows 2-days-old *A. laboriosa* worker bees flying out of hive No. 4. Of the 60 workers present in that colony, 15 (25%)flew out. The bees did not fly from the entrance upward like *A. mellifera*, but downwards, and some dropped on the ground. Returning bees did not direct them to the entrance, but tried to enter the hive from the bottom. Because they did not succeed in entering, they flew out several times trying to reenter from the bottom. Therefore, the authors located a box beneath the hive, which made flight beneath the bottom board of the hive impossible. In this case, many bees landed on the box 5 - 15 cm away from the entrance and then walked to it. At the age of seven days, 70% of *A. laboriosa* bees flew out of the hive. As the workers aged, more of them returned directly to the entrance. However, at the age of 14 days, the majority still did not return directly to the entrance, but landed several cm apart and then walked to the entrance.

During overcastted days, young *A. laboriosa* workers did not fly, although *A. mellifera* foragers were flying.

Fig. 3B shows that young *A. laboriosa* workers in *A. mellifera* colony did not fly out of the hive within a short period of several minutes, characteristic of the periodic mass flights of young worker bees of this species (Woyke et al 2003). On the contrary, the young *A. laboriosa* workers flew out of one colony within several hours. *A. mellifera* workers from the same colony performed orientation flights on 31 December, 5 and12 January at 1230-1330 h, 1230-1315h and 1355-1425h, respectively. No extraordinary higher flight activity workers was observed in *A. laboroisa* workers at this time, during the three days presented at fig. 3B, nor during the other nine days at which *A. laboriosa* workers were flying.

Guard duties of *A. laboriosa* workers at nest entrance of *A. mellifera*

A. laboriosa workers guarding the hive entrance of the *A. mellifera* colony were first recorded at the age of 11 days. Next, they were observed on all the other days. *A. laboriosa* guard bees inspected incoming *A. mellifera* bees with their antennae and front legs. Three positions were observed. *A. laboriosa* inspected incoming foragers with the head directed outside the entrance. However, very often it was with the head directed to the entrance. *A. laboriosa* also walked at the frontage of the bottom board before the entrance and inspected incoming bees directed head up.

DISCUSSION

A. mellifera did not accept *A. laboriosa* introduced directly into *A. mellifera* colonies. However, it took care of *A. laboriosa* introduced into small cages. *A. mellifera* also accepted *A. laboriosa* workers when they were previously familiarized in *A. mellifera* colonies. This indicates that *A. mellifera* uses environmental cues acquired by *A. laboriosa* bees in *A. mellifera* colonies, to accept the other species. Similarly, *A. dorsata* was accepted by *A. mellifera* (Woyke et al., 2000 and 2001), as well as by *A. cerana* (Woyke et al., 2002). However, *A. dorsata* uses heritable cues in recognition and rejection of adults of the other species. The success of introduction of *A. laboriosa* bees into *A. mellifera* colonies enabled the study of some behavioral features in changed conditions. *A. laboriosa* workers already started to fly from the hive at the age of two days. However, they used the flight pattern of workers living in the free hanging nests and therefore, it had

difficulty finding the hive entrance. On the other hand, young *A. laboriosa* workers in *A. mellifera* hives did not show any sign that they would perform one to three short periodic mass flights characteristic of workers in free hanging *A. laboriosa* nests (Woyke et al., 2003). However, young *A. laboriosa* bees did not fly while the sky was overcast the way like the free living bees did not perform their periodic mass flights (Woyke et al., 2003), although older bees of both species were foraging in those conditions. *A. laboriosa* workers learned in *A. mellifera* colonies the template cue of the colony and used it as guard bees to check entering *A. mellifera* foragers.

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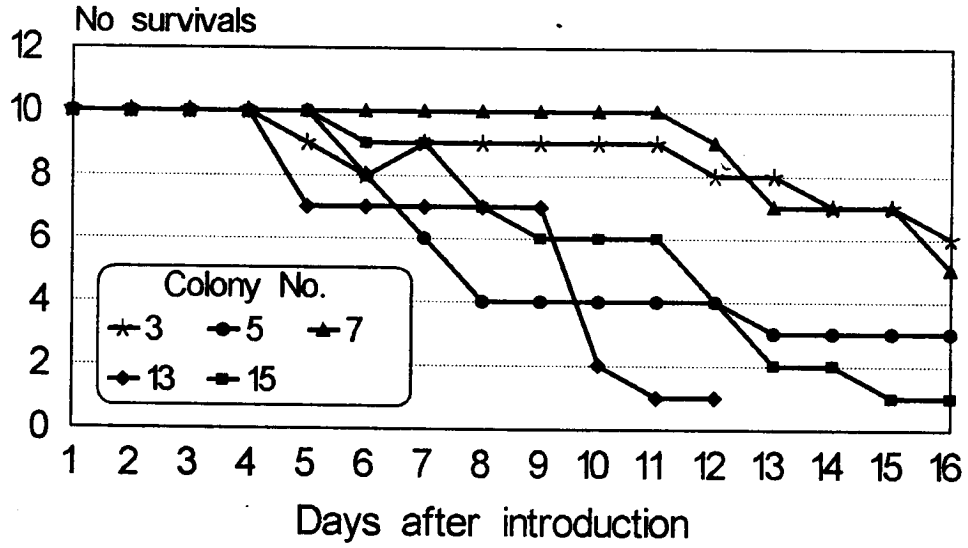
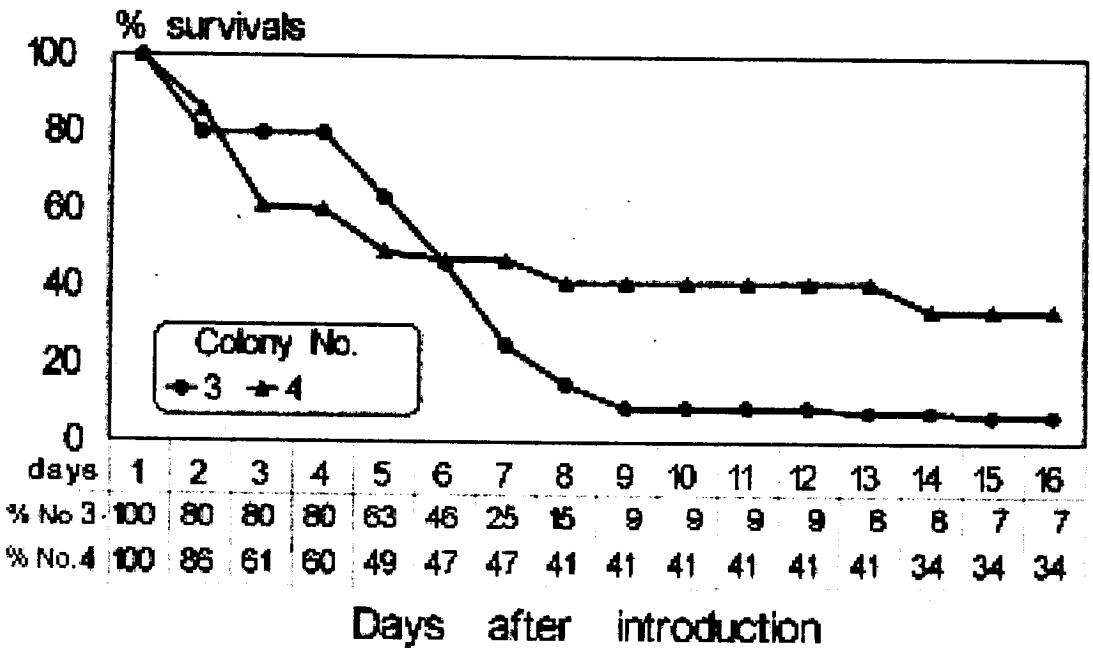


Fig. 1. Survival of *A. laboriosa* workers in small cages in five *A. mellifera* colonies (per 10 workers in each cage).



days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
% No 3	100	80	80	80	63	46	25	15	9	9	9	9	8	8	7	7
% No.4	100	86	61	60	49	47	47	41	41	41	41	41	41	34	34	34

Fig. 2. Percentage survival of *A. laboriosa* workers introduced into two *A. mellifera* colonies and released the next day (106 workers in colony 3 and 70 in colony 4).

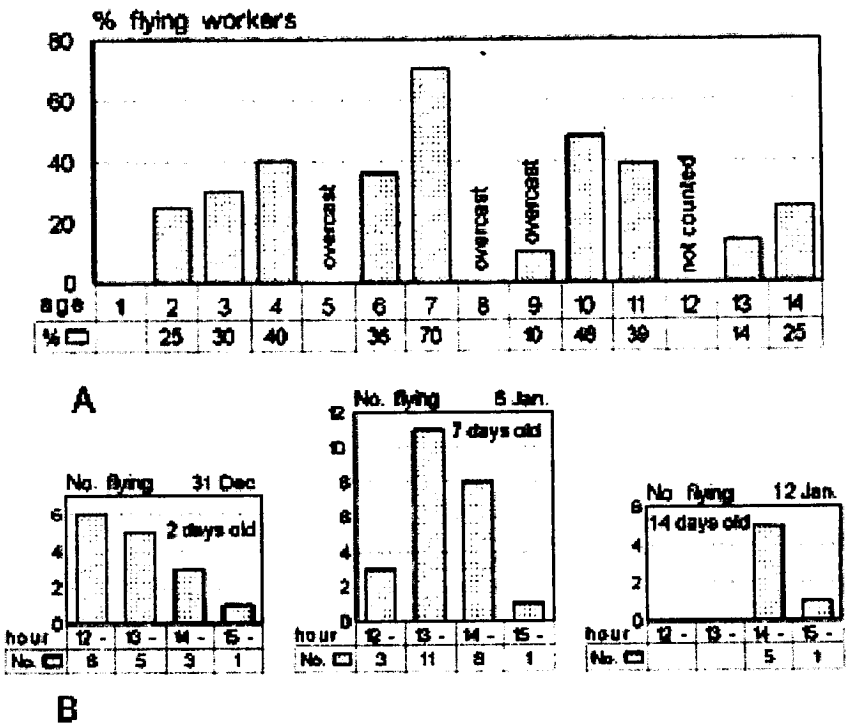


Fig. 3. A. Percentage of *A. laboriosa* workers, (of those present in the colony), flying at different age (days) out of *A. mellifera* hive No. 4.
B. Number of *A. laboriosa* workers flying at different day time, out of *A. mellifera* hive No. 4.

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