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First evidence of hygienic behaviour in the dwarf honey bee Apis florea

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Abstract The hygienic behaviour of eight *A. florea* honey bee colonies was investigated in Thailand. Sealed brood was deep frozen or killed using entomological pins. The pins were 0.2 or 0.7 mm in diameter. The removal of killed brood by the bees was checked in intervals ranged from 24 to 60 h after it had been presented to the colonies. Almost eighty nine (89%) of the freeze-killed brood was removed by the bees within 24 h after being introduced into the combs, while 95% of the pin-killed brood was removed within 48 h after introduction. Thus, the *A. florae* colonies could be recognized as hygienic honey bees.

Keywords *Apis florea*, hygienic behaviour, freeze-killing, pin-killing, migratory honey bees

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NOTES AND COMMENTS

First evidence of hygienic behaviour in the dwarf honey bee *Apis florea*

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Both of the (hive) cavity-nesting honey bees *Apis mellifera* and *A. cerana* display hygienic behaviour which is seen by the removal of dead brood parasitized by *Varroa destructor* or killed by bacterial diseases (Spivak, 1996; Evans and Spivak, 2010). To the contrary, Woyke (1996) and Woyke *et al*. (2004) found that the migratory open-air nesting honey bees *A. dorsata* and *A. laboriosa* do not open sealed cells containing dead brood killed by the parasite *Tropilealaps clareae* or by any other disease. It is believed that this behaviour prevents the spread of parasites and diseases inside the nest. The third migratory open-air nesting dwarf honey bee, *A. florea* is parasitized by the mite *Euvaroa sinhai* (Akratanakul and Burgett, 1976; Ahmadi, 1988) and is infected by some fungi (Alizadeh and Mossadegh, 1994). Woyke *et al.* (2011) suggested that *A. florea* removes dead brood, but no data were presented. In the present study we therefore investigated the hygienic behaviour of the dwarf bee *A. florea*.

Fig. 1. Freeze-killed brood inserted in the comb of *Apis florea* colony No. 3, 9 March 2011, Ratchaburi, Thailand.

The investigation was carried out in Thailand, at the Ratchaburi campus of King Mongkut's University of Technology in Thonburi (13º 59' N, 99º 51' E), from 9 to 13 March 2011. Eight *A. florea* colonies were investigated. Three colonies were naturally established on the campus while five colonies were transferred from an area 1.5 km away. The investigation of the transferred colonies started 5 days after they were introduced on campus. Comb pieces of sealed brood were deep freeze-killed (Fig. 1) or pin-killed using pins with 0.2 mm or 0.7 mm in diameter. The removal of the killed brood was checked in time intervals between 24 and 60 h after it had been presented to the bees. One-way ANOVA was performed on the data concerning the 24 hours interval and the two-way ANOVA on data concerning results of pins of two diameters in three intervals. For statistical calculations, the percentages of the remaining killed brood were transformed according to the arcsine function. Correlation coefficients were also calculated.

Table 1 summarizes all data concerning percentages of sealed brood killed by the three methods which remained in the cells after different time intervals. Established colonies had clearly removed higher percentage of killed brood in 24 hours than the transferred ones. In particular, the established colonies had less than 5% of freeze-killed sealed brood left 24 h after the brood was reinserted into the combs.

A one-way ANOVA of the results of all 8 colonies, showed that 24 h after killing the brood, the method of killing (frozen, or killed by pins of 0.7 or 0.2 mm) significantly affected the percentage of the remaining sealed brood ($F_{5, 23}$ = 13.30, P = 0.000). Significant differences were also detected between the established and the transferred colonies. In particular, 5.5, 5.3, and 6.2 times significantly more brood, killed by the three methods, was left in the transferred colonies than in the established ones. The Duncan's multiple range tests, however, did not show significant differences between the means of the remaining brood killed by the three methods in the established colonies. No significant difference was also detected between percentages of left freeze-killed brood or pin-killed brood with the thick pin of 0.7 mm

Table 1. Remains of sealed brood killed by three methods in *A. florea* colonies. Different capitals (A, B, C) indicate significant differences ($p < 0.05$) between means in the same column, concerning the method of killing the brood. Different small letters (a, b, c) indicate significant differences ($p < 0.05$) between means in the same row, concerning the time of checking the brood.

Method of killing	No combs	No cells	Mean % sealed cells left after hours			
			24	42	48	60
Established colonies						
Frozen E	3	376	3.5Aa	-	0.0	-
Pin 0.7 E	3	150	4.0Ab	0.7Aa	0.0	0.0
Pin 0.2 E	3	150	6.7Ab	2.0Aa	0.0	0.0
Transferred colonies						
Frozen T	5	807	19.4Ba	-	0.0	-
Pin 0.7 T	5	250	21.2Bb	10.2Ba	4.0Aa	0.0
Pin 0.2 T	5	250	41.4Cc	15.6Cb	5.6Aa	0.0

diameter within the transferred colonies, but twice as much brood remained after being pierced with the thin, 0.2 mm pin (Table 1). Thus, the bees from the transferred colonies removed brood pinned with the thin pin much less effectively than pinned with the thick pin or freeze-killed. The same effect of the pin difference in diameter is shown also later, after 42 h ($F_{3, 15} = 15.10$, $p = 0.000$). Forty-eight hours after killing, no killed brood remained in the established colonies, while a very small proportion of pin-killed brood (4.0-5.6%) still remained in the transferred colonies. The removal time of the killed brood was affected by the method of killing. All frozen brood in the 8 colonies was removed within 48 h after being introduced into the combs (Fig. 2).

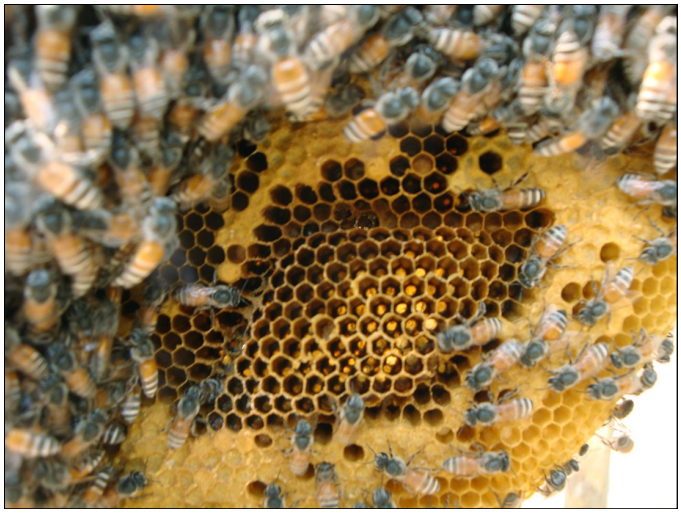

Fig. 2. Empty piece of comb 48 h after insertion of freeze-killed brood into colony No. 3.

A two-way ANOVA on the data concerning results of pins of two diameters in three intervals, showed that diameter of the pins ($F_{1, 29} = 9.43$, $p = 0.005$) as well as the checking time ($F_{2, 29} = 34.17$, $p = 0.000$), significantly affected the percentage of brood which had been left. No significant interaction was found between the two factors ($F_{2, 29} = 2.25$, $p = 0.127$). In the established colonies, 98.0-99.3% of pin-killed brood was removed within 42 h and all killed brood was removed within 48 h. In the transferred colonies 94.4-96.0% of pin-killed brood was removed within 48 h and all killed brood was removed within 60 h (Table 1). The correlation between the percentages of brood left in the 5 transferred colonies, after being pierced with the thick or the thin pin (0.7 mm or 0.2 mm), was significant; $r = 0.95$, $df = 4$, $p = 0.013$. However, no significant correlation was found between the percentage of the remaining freeze-killed brood and the percentage of remaining brood pierced with either the thick pin (0.7 mm; $r = 0.16$, $df = 4$, $p = 0.800$) or the thin pin (0.2 mm; $r = 0.26$, $df = 4$, $p = 0.676$).

The slower removal of dead brood by the transferred colonies than by the established ones was unexpected. No proven explanation exists as to whether this was just variation or whether two populations of different hygienic behaviour were present, or perhaps that the transportation affected the results. Further investigations are necessary. In conclusion, all 8 colonies removed 89% of the freeze-killed brood within 24 h after it had been inserted into the combs and 95% of the pin-killed brood within 48 h. Thus, those *A. florea* colonies have to be considered as hygienic honey bees. Further investigations are desirable concerning the variation of hygienic behaviour of different populations of *A. florea*, as well as the behaviour in shorter intervals of 8 and 16 h, which could give clearer and more profound results.

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