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Anti-predator Fan-blowing in Guard Bees, *Apis mellifera capensis* Esch

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Abstract Honeybees employ different defensive strategies depending on the nature of potential predators. The Cape honeybee, *Apis mellifera capensis*, exhibits a unique fan-blowing behaviour to repel ants and similar sized insects at the nest entrance. Guard bees turn in alternating clockwise and anticlockwise circles on a fixed vertical axis and fan their wings when encountering tramp ants (*Pheidole megacephala*), aphids (*Myzus persicae*) and termites (*Trinervitermes trinovoides*) on the landing board of a hive. The blowing force was constant and was driven by fanning with a wing-beat frequency of 274.8±16.3 Hz, which exceeds that of flight. On the contrary, small hive beetles (*Aethina tumida*) were removed by mauling and expulsion whereas larvae of the greater waxmoth (*Galleria mellonella*) and the mealworm (*Tenebrio molitor*) were seized with mandibles and thrown from the nest area.

Keywords *Apis mellifera capensis* · predators · fan-blowing · wing-beat frequency

Introduction

The behaviour of honeybees reflects the persistent trait of deterring and repelling serious threats to the colony, including invasions by pedestrian predators (Breed et al. 2004; Koeniger et al. 2004; Duangphakdee et al. 2005). However, different

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defensive strategies are employed by honeybees depending on the nature of the potential predator as evidenced by heat-balling hornets (Ken et al. 2005), group mauling small hive beetles (Ellis and Hepburn 2006), and simple, unidirectional fanning against ants (Spangler and Taber 1970; Seeley et al. 1982). Although Spangler and Taber (1970) reported a then undescribed form of fan-blowing behaviour in European-derived honeybees in the USA, we observed a more complete form of this behaviour exhibited by the Cape honeybee, *Apis mellifera capensis,* which is directed at ants and similar sized insects. The cosmopolitan tramp ant, *Pheidole megacephala,* is a common predator on honeybees in Africa and their invasions of our colonies stimulated new experiments on the defensive strategies of honeybees. When confronted by these ants, the guard bees at the hive entrance perform a series of clockwise and anticlockwise turns which we designate as “fan-blowing”, which very effectively blows such ants away. Here, we report the results of experiments to assess the effectiveness of fan-blowing.

Materials and Methods

Test Animals

Observations of different defensive strategies of guard bees were conducted on five colonies of *A. m. capensis,* at Grahamstown, South Africa. Tramp ants, *P. megacephala,* nesting close by the colonies were recruited to a trail of syrup drops onto the landing boards of the bee hives. To test the possibility that ant volatiles might alert the guard bees, pieces of filter paper on which ants were squashed were placed on the landing boards. One cm^2 squares of standard Whatman’s filter paper were placed lying flat on the landing board and a single ant caught with forceps was immediately squashed on it. This was repeated ten times. In addition, different kinds and sizes of insects, two pests of honeybees (wax-moth larvae, *Galleria mellonella*; small hive beetles, *Aethina tumida*) and three neutral ones (mealworms, *Tenebrio molitor*; termites, *Trinervitermes trinovoides*; aphids, *Myzus persicae*) were used to assess the kinds of defensive strategies the honeybees might use in addition to the fan-blowing behaviour against ants that we observed.

Fan-blowing and Wing-beat Frequency

For all observations and tests, ten individual fan-blowing guard bees from each colony were recorded on video. The numbers of frames in each fanning circle and the sequence and frequencies of clockwise and anticlockwise circles were counted. We used a Sony MZ-NH1 minidisc recorder and a Sony ECM-719 stereo-microphone to record the buzzing sounds of the fan-blowing bees. These results were processed with the software programme ‘wavesurfer 1.8.5’ (Sjolander and Beskow 2008) which converts such sounds into wing-beat frequency. The wing-beat frequencies of bees engaged in normal fanning to cool the hive (n=3 colonies; ten bees per colony) and foragers fanning at a feeding dish (n=10 foragers) were also measured.

Fan-blowing Intensity

To determine whether fan-blowing intensity was constant as the bees turned in their circles, we placed an oiled sheet of plastic on the landing board and sprinkled powdered candle dyestuff (Candlemakers Supplies, UK - http://www.candlemakers.co.uk) just in front of a guard bee. When the bee turned during fan-blowing such that the abdomen now pointed outwards, it blew the dyestuff on the plastic sheet. If fan-blowing intensity is constant, the pattern formed must form a semi-circle because, as the bee turns, its vertical axis remains virtually at the centre of the circle in which it performs. To determine the effectiveness of fan-blowing ants away, an oiled sheet of plastic graduated in cm lines was placed on the landing board and the point at which the ants came to rest was marked and a frequency distribution for distance was determined.

Statistical Analysis

Analysis of variance (ANOVA) was used to test for differences in the numbers and duration of the circles among the five colonies. A dependent sample t-test was used to compare the mean number of clockwise and anticlockwise circles. ANOVA tests were also used to test for differences in wing-beat frequency among the five colonies for fan dances and three colonies for normal fanning. A single sample t-test was used to compare the mean wing-beat frequency of the fan dance with that of normal fanning and flight. Homogeneity of the variances between groups was checked using Levene's test, and a log transformation of the data was used to stabilise the variances where necessary. Differences in the circle frequencies and the wing-beat frequencies for ten fanning bees within each colony were tested using chi-square tests. The means and standard deviations of each variable were calculated. We used Statistica 8.0 software to analyse the data (StatSoft 2007).

Results

When guard bees detected ants on the landing board, they began fan-blowing their wings, turning in clockwise and anticlockwise circles, and blowing the ants away (cf. electronic attachment 1). The video clips confirm that when a bee turns while fan-blowing, it remains virtually at the centre of the circle through which it moves (Fig. 1). The effect of fan-blowing is analogous to a commercial leaf-blower. The mean distance that ants were blown away by fan-blowing bees was 3.57 ± 2.90 cm with a range of 1 to 15 cm (n=251 ants, Fig. 2). Direct evidence that ants were indeed blown off the landing board is provided by the fact that when we placed a bowl of water just below the landing board, several hundred ants were collected in this way by the end of the day. There was no evident reaction by guard bees to the pieces of filter paper with squashed ants.

Ants often attempted to grasp a bee and several ants were observed to kill a bee in 1/~500 encounters (cf. electronic attachment 2). In colonies experiencing heavy attacks by ants, the bees move above the entrance fan-blowing vertically downwards. Tests with different kinds and shapes of insects demonstrated that

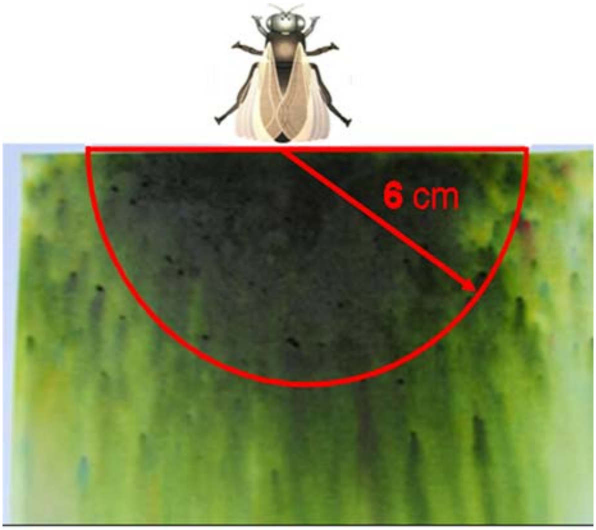

Fig. 1 Blown dyestuff pattern demonstrates constancy of wing-beat frequency during fan-blowing. Area within curved line is 56.5 cm^2.

aphids, ants and termites of similar size, were all blown away by the fan-blowing bees. The larger larvae were seized by the guard bees and thrown from the landing board, whereas small hive beetles were mauled by several guards and expelled from the landing board.

The numbers and the directions (clockwise or anticlockwise) of the circles were recorded. Ten guard bees from each of five colonies performed 536 circles averaging 10.72±4.97 circles per session. There were significant differences in performances within colonies except for colony 5 (chi-square test: colony 1: χ_9^2=25.5, p=0.002; colony 2: χ_9^2=36.0, p<0.001; colony 3: χ_9^2=17.9, p=0.036; colony 4: χ_9^2 =18.8,

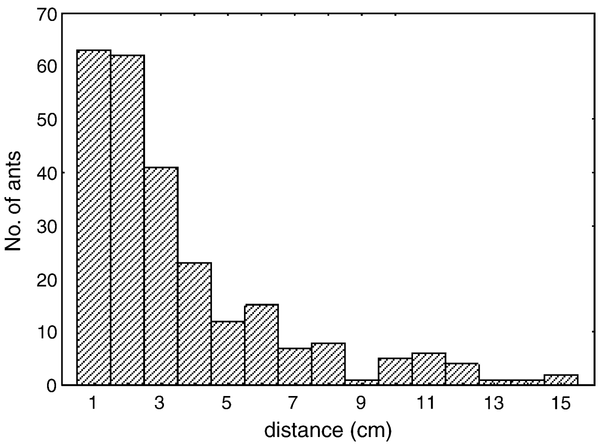

Fig. 2 Frequency distribution of the distances (cm) ants (*Pheidole megacephala*) were blown by honeybees (*Apis mellifera capensis*), n=251.

p=0.026; colony 5: χ_9^2=7.1, p=0.628), but not between colonies (ANOVA: $F_{4,45}$= 0.78, p=0.548; Levene: $F_{4,45}$=0.63, p=0.646). The five colonies averaged 5.5±2.8 clockwise and 5.2±2.5 anticlockwise circles and there was no significant difference between them (dependent t-test: t_{49}=0.89, p=0.379). However, when the sequence and frequencies of clockwise direction were counted, significantly more bees (64%) began their fan dances in a clockwise than in an anticlockwise direction (chi-square test: χ_1^2=3.9, p=0.048)

Ten bees from each colony made a total of 272 clockwise circles and 264 anticlockwise circles, and the average of the former was 0.74±0.31 sec and 0.73± 0.30 sec for the latter. Duration did not differ significantly for the two types of circles (dependent t-test: t_{263}=0.08, p=0.933), nor among the five colonies (ANOVA: clockwise circles: $F_{4,267}$=1.5, p=0.191; Levene: $F_{4,267}$=2.2, p=0.073; anticlockwise circles: $F_{4,259}$=2.2, p=0.074; Levene: $F_{4,259}$=0.24, p=0.913).

Wing-beat frequency for 10 fan-blowing bees from each colony averaged 274.8± 16.3 Hz with no significant differences among the colonies (ANOVA: $F_{4,45}$=0.67, p=0.615; Levene: $F_{4,45}$=3.1, p=0.024), nor within them (chi-square test: colony 1: χ_9^2=13.6, p=0.137; colony 2: χ_9^2=3.8, p=0.923; colony 3: χ_9^2=1.4, p=0.998; colony 4: χ_9^2=10.5, p=0.312; colony 5: χ_9^2=15.2, p=0.086). The mean wing-beat frequency for normal fanning for three colonies was 181.1±12.2 Hz. There were no significant differences among the three colonies (chi-square test: colony 1: χ_9^2=9.3, p=0.424; colony 2: χ_9^2=7.7, p=0.565; colony 3: χ_9^2=5.1, p=0.829).

The average wing-beat frequency for fan-blowing is significantly higher than in normal fanning for cooling (t-test: t_{49}=40.7, p<0.001) and in normal flight (t-test: t_{49}=19.5, p<0.001; normal flight mean: 230 Hz - Altshuler et al. 2005). The wing-beat frequency of the foragers fanning at the feeding dish was 270.1±9.3 Hz, which is also significantly higher than that in normal flight (one-sample t-test: t_9=13.6, p<0.001) and normal fanning to cool the hive and 'sterzeln' to spread Nasonov pheromones (one-sample t-test: t_9=30.2, p<0.001). There was no significant difference between the wing-beat frequency of fan-blowing bees from that of foragers fanning at the feeding dish (t-test: t_{58}=0.89, p=0.376).

In another experiment, we recruited both honeybees and ants to the same feeder dish. At this site the bees on the dish also fan-blowed against them, but without any turns and circles. As a result, they could only blow away ants behind them while those in front could still suck syrup. But, at the same time that the bees were blowing ants they were simultaneously imbibing syrup themselves (cf. electronic attachment 3).

Discussion

It could be argued that predator repellence might be the goal of fan-blowing in which case the possibility of Nasonov scenting needs to be considered. However, the primary constituents of this secretion, citral and geranic acid, are well known scents commonly used in perfumery (cf. Free 1987), were not detectable during our observations on fan-blowing bees. Conversely, Spangler and Taber (1970) suggested that volatiles emanating from ants signalled their presence to bees, but in our experiments, scent alone played no apparent role in the guard bee/ant interaction.

The numbers, direction and duration of the circles turned during fan-blowing differed within but not among colonies. There was no significant difference between the mean number of clockwise and anticlockwise circles performed, but more bees (64%) began fan-blowing in a clockwise direction. The question of clockwise or anticlockwise turning during fanning could be simply dependent on the conditions near the nest such as on cues of the ants (in which direction of the fanning bee they approach) or by parameters associated with ambient illumination. There were neither significant differences within nor among colonies for wing-beat frequency during fan-blowing. The dyestuff pattern confirmed that wing-beat frequency in fan-blowing is constant.

The wing-beat frequency of the fan-blowing (274.8±16.3 Hz) and foragers blowing ants at a feeding dish (270.1±9.3 Hz) did not differ significantly; however, both are significantly greater than in normal flight (230 Hz - Altshuler et al. 2005) and in fanning for cooling (181.1±12.2 Hz). Because there is a linear correlation between metabolic power output and wing-beat frequency (Feller and Nachtigall 1989), fan-blowing may be energetically the most costly activity produced by bees when the thoracic musculature of honeybees is physiologically coupled to the wings (i.e. when the mechanical coupling between thoracic muscles and wings is switched off, then greater energy is expended as in heat-balling—Ken et al. 2005).

Given the relatively small size of ants against bees and the fact that the former may well attack the latter, fan-blowing is an extremely effective method of repelling ants. Adding to this the observations of Spangler and Taber (1970), this behaviour may well be widespread, but seldom observed, throughout the races of *Apis mellifera*. At a feeding dish the bees simply blow ants behind them without turning. This is somewhat surprising because, although the amount of energy expended in fan-blowing and ant-blowing is virtually the same, fan-blowing is clearly a far more effective method of dispersing ants. However, the video clips clearly show that the bee foragers are actually imbibing syrup when simultaneously blowing ants.

But why do the bees turn in circles instead of turning around with their abdomens facing the ants and fan-blowing in a stationary position which would appear more directed than circular fanning? Possibly for two reasons: although honeybees are more sensitive to moving objects than to stationary ones (Lehrer 1997), when ants detect fan-blowing bees, they usually crouch to the substrate and only begin to move again once the guard bees have stopped fan-blowing. Because ants can move much faster on foot than guard bees, they can easily escape or even steal into the hives if guard bees are not vigilant. Alternatively, the bees may use the principle of re-afference to detect stationary ants.

The principal advantage of fan-blowing in circles is that bees can enlarge their area of counter-attack so reducing the chance of ants passing through into their hives. The ants also try to bite the legs of bees as they turn in clockwise and anticlockwise circles so that reversing direction might well reduce the chance of being caught by ant stealth. Fan-blowing is a well-adapted behaviour suggestive of yet another example of a co-evolutionary arms race.

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