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Apidologie (2012) 43:195–200

DOI: 10.1007/s13592-011-0098-5

Original article

Cooperative wasp-killing by mixed-species colonies of honeybees, *Apis cerana* and *Apis mellifera*

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Received 4 April 2011 – Revised 8 August 2011 – Accepted 18 August 2011

Abstract – The cooperative defensive behaviour of mixed-species colonies of honeybees, *Apis cerana* and *Apis mellifera*, were tested against a predatory wasp, *Vespa velutina*. When vespine wasps hawk honeybees at their nest entrances, the difference in the numbers of bees involved in heat-balling among pure species and mixed-species colonies was not significantly different. However, in the mixed colonies, the numbers of *A. cerana* and *A. mellifera* workers involved in heat-balling were significantly different. The duration of heat-balling among these three groups was significantly different. During heat-balling, guard bees of both species in mixed colonies raised their thoracic temperatures and the core temperatures of the heat-balls were about 45°C, which is not significantly different from that of the pure species. These results suggest that the two species of honeybees can cooperate in joint heat-balling against the wasps, but *A. cerana* was more assertive in such defence.

cooperation / defensive behaviour / mixed species / *Apis cerana* / *Apis mellifera*

1. INTRODUCTION

Altruism and cooperation are the main issues that make honeybees different from solitary insects; but, cooperation in social organisms has been a difficult issue for evolutionary theory since the time of Darwin (Hamilton 1963; Axelrod and Hamilton 1981). The evolutionary distance between *Apis cerana* and *Apis mellifera* is sufficiently small that transfers of pupae between *A. cerana* and *A. mellifera* colonies result in the acceptance of newly eclosed workers (Atwal and Sharma 1968; Tan et al. 2006), enabling the construction of artificial mixed-species colonies. Using an *A. cerana* and *A. mellifera* mixed-species model as a probe for these theories, it is also possible to define elements of behaviour that probably pre-date, and others that followed speciation. This approach has allowed discoveries such that these two species can mutually interpret their dance languages (Su et al. 2008; Tan et al. 2008), cooperate in queen retinue behaviour (Yang et al. 2010a), comb building (Yang et al. 2010b), and colony thermoregulation (Yang et al. 2010c).

The proximate mechanisms of defensive behaviour used by honeybees against wasp predation are well understood. *Vespa velutina*, a wasp endemic to southeast Asia, preys on honeybees, both the native *A. cerana* as well as the introduced European *A. mellifera* (Matsuura and Yamane 1990; Tan et al. 2005; Ken Tan et

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Manuscript Editor: David Tarpy

al. 2007). When under wasp attack, the two honeybee species adopt different defence strategies: *A. cerana* workers recruit nestmates by shimmering vigorously and shaking their abdomens from side to side accompanied by a peculiar hissing (Sakagami 1960). While workers of *A. mellifera* do not shimmer, they do recruit nestmates to block the nest entrances and then to attack the wasps directly, biting and stinging them (Heinrich 1979; Stabentheiner et al. 2002). Finally, guard bees of both species kill the wasps by heat-balling. The lethal thermal limit for the wasp is about 45.7°C, which is lower than that for *A. cerana* and *A. mellifera* (50.7°C and 51.8°C, respectively) (Tan et al. 2005). Thus the wasps can be killed using this marginal temperature difference by heat-balling and asphyxiation by carbon dioxide poisoning (Ono et al. 1987; Tan et al. 2005; Sugahara and Sakamoto 2009). Because such behaviour as heat-balling but not shimmering is common to both species the former was very probably conserved after speciation. Here we test colony heat-balling behaviour in mixed-species colonies using the wasp as the stimulus and set the following questions: (1) Do the two species cooperate in heat-balling? If so, (2) How do the two species behave?

2. MATERIALS AND METHODS

Six queenright *A. cerana* and six *A. mellifera* colonies were placed in an apiary on the campus of Yunnan Agricultural University, Kunming, China. The *A. mellifera* were commercial Chinese stock primarily of *Apis mellifera ligustica* origin. The *A. cerana* were typical of wild colonies in Yunnan Province, China. In September c, we equalized the colonies in Langstroth hives such that each contained 2 frames of brood and 2 of honey and pollen. We then reciprocally transferred a single frame of eclosing brood to form 6 mixed-species colonies, 3 headed by an *A. cerana* queen and the other 3 by an *A. mellifera* queen. The result was that each experimental colony consisted of 2/3 of the host species and 1/3 of introduced species. Three other colonies each of *A. cerana* and *A. mellifera* were left as unmanipulated controls.

Defensive behaviour against wasps was investigated when the newly emerged workers were about three weeks old, the peak age of defensive behaviour (Seeley 1985). Pure *A. cerana* and *A. mellifera* colonies with the same age cohorts of workers were selected as control groups. For the bioassays, a live wasp, *V. velutina*, was tied at the petiole with fine wire such that the wasp could fly and move freely within the length confines of the wire. Its movements could alert the guard bees to form a heat-ball. A thermometer (Sensorted, BAT-12, with a resolution of±0.1°C) with an external sensing probe was used to measure the core temperature of the heat-ball. Each wasp was used only once on a colony so that each colony received a new wasp. We recorded the numbers of bees of each species during heat-balling, the duration of heat-balling episodes, the core temperature in the heat-balls, and the numbers of dead bees associated with each heat-balling incident. The performances of the workers during heat-balling were recorded (n=9 heat-balls per colony type) on video (Panasonic NV-GS400GC).

The numbers of honeybees in heat-balling, the duration of heat-balling, deaths during heat-balling and the core temperature differences between mixed-species colonies and control groups were compared using ANOVA procedures. Tukey post-hoc multiple comparison tests were used to test for significant group effects (Johnson and Wichern 2002). The means and standard deviations of each variable were calculated. All tests were performed using Statistica (StatSoft Inc 2009).

3. RESULTS

3.1. Comparisons between pure and mixed-species colonies

The core temperatures of the mixed-species heat-balls formed by the two species did not differ significantly from that of pure colonies (ANOVA: $F_{2,\ 24}$=0.30, P=0.745; Table I). The numbers of honeybees involved in heat-balling in *A. cerana* (77.3±23.0), *A. mellifera* (69.8±31.1) and mixed-species colonies (62.6±15.2)

Table I. Mean±SD of the number of honeybees in heat-balls, duration, core temperature and death during the heat-balling (n=9 heat-balls per colony type).

Colony type	No. of honeybees	Duration (min)	No. of dead honeybees	Core temperature (°C)
Pure *A. cerana*	77.3±23.0a	37.0±8.7a	2.8±2.7a	45.4±1.0a
Pure *A. mellifera*	69.8±31.1a	24.2±14.1b	9.5±6.7b	45.5±1.2a
Mixed	62.5±15.2a	25.0±4.2b	4.1±1.8a	45.8±0.8a

Means within one column followed by a different lowercase letter are significantly different (Tukey multiple comparisons, $P<0.05$).

were not significantly different (ANOVA: $F_{2,\ 24}$= 0.47, P=0.630; Table I). The durations of heat-balling in *A. cerana* (37.0±8.7 min), *A. mellifera* (24.2±14.1 min) and the mixed-species colonies (25.0±4.2 min) were significantly different (ANOVA: $F_{2,\ 24}$=4.74, P=0.018; Table I). Post-hoc tests showed the duration in pure *A. cerana* colonies was significantly longer than that in pure *A. mellifera* and mixed-species colonies.

The numbers of dead honeybees recovered after heat-balling in *A. cerana* (2.8±2.7), *A. mellifera* (9.5±6.7) and mixed-species colonies (4.1±1.8) were significantly different (ANOVA: $F_{2,\ 24}$=6.29, P=0.006; Table I). Post-hoc tests showed the number of dead honeybees recorded after heat-balling in *A. mellifera* was significantly higher than that in pure *A. cerana* and mixed-species colonies.

3.2. Comparisons within mixed-species colonies

In mixed-species colonies the number of *A. cerana* workers (48.1±14.7) involved in heat-balling was significantly greater than *A. mellifera* (14.4±7.6) (Paired t-test: t_8=5.67, P< 0.001). The proportion of dead *A. cerana* workers (7.6%±4.0%) recorded after heat-balling was significantly greater than the proportion of dead *A. mellifera* workers (5.4%± 5.1%) (Paired t-test: t_8=2.81, P=0.023).

4. DISCUSSION

It could be objected that because the mixing of two species which do not occur as a sympatric autochthon in the same habitat, this investigation could be regarded as an experimentally designed artefact. However, in any experiment, some variable of interest is held constant which, ipso facto, makes it contrived and artefactual. We specifically used the approach taken to assess the interspecific gap between the both species in behavioural terms, in which case it is simply another experiment.

Although heat-balling wasps is well documented (Ono et al. 1987; Tan et al. 2005), comparisons of behavioural sequences during heat-balling such as attracting additional recruits to the guard bee cohort, increased numbers of guard bees that raise thoracic temperature to heat-balling a wasp until it is killed have not been previously measured for *A. cerana* and *A. mellifera* colonies. In our observations the core temperature and numbers of bees involved in heat-balling in mixed-species colonies did not significantly differ from either of the pure species colonies or mixed colonies (Table I). These results indicate that there is a similar evolutionary basis for combined nest defence against wasps in two, sister honeybee species.

However different honeybee species have different risk assessments, resulting in different thresholds for response (Gordon 1996; Jones et al. 2004). *A. cerana* is generally reported as being mild, tolerant and timid in defence behaviour (Ruttner 1988); however, they show a number of behavioural patterns which prove to be very effective against traditional enemies. One of the most striking traits is group defence. For example, if attacked by powerful enemies such as wasps and hornets, *A. mellifera* counterattacks, but *A. cerana* rather retreats into the nest (Schneider and Kloft 1971).

In our observations, we found in all mixed colonies that when a wasp was placed close to the entrance, *A. mellifera* workers initiated the attack first, though by single individuals, and then *A. cerana* bees joined and formed a mixed species ball. The number of *A. cerana* involved in heat-balling was much greater than *A. mellifera*, which indicates that *A. cerana* workers in these mixed colonies played a more active part than *A. mellifera* workers in heat-balling. Of course, the higher number of *A. cerana* workers engaged in heat-balling could reflect a higher proportion of this species in the mixed colonies.

In the bee balls of mixed-species and *A. mellifera* colonies the duration times were 25 and 24.2 min respectively, and in the case of pure *A. cerana* was 37 min. This apparent anomaly is explained by the fact that *A. mellifera* workers are about 30% heavier than *A. cerana* workers so, in heat-balling, the former are probably more efficient in killing wasps. Hence the duration of heat-balling by *A. mellifera* and mixed colony bees is also about 30% shorter than *A. cerana*.

Moreover, in the mixed balls, *A. mellifera* bees were at the outer edge of the ball while *A. cerana* bees formed a tight inner core. The sequence of photographs in Figure 1 illustrate ball forming, which is obviously determined by their specific genetics (Gordon 1996). We found that *A. cerana* workers are more aggressive than *A. mellifera* workers. The pure *A. mellifera* colonies lost more individual guard bees than *A. cerana* colonies in the heat-balling, but in the mixed colonies, *A. cerana* suffered heavier

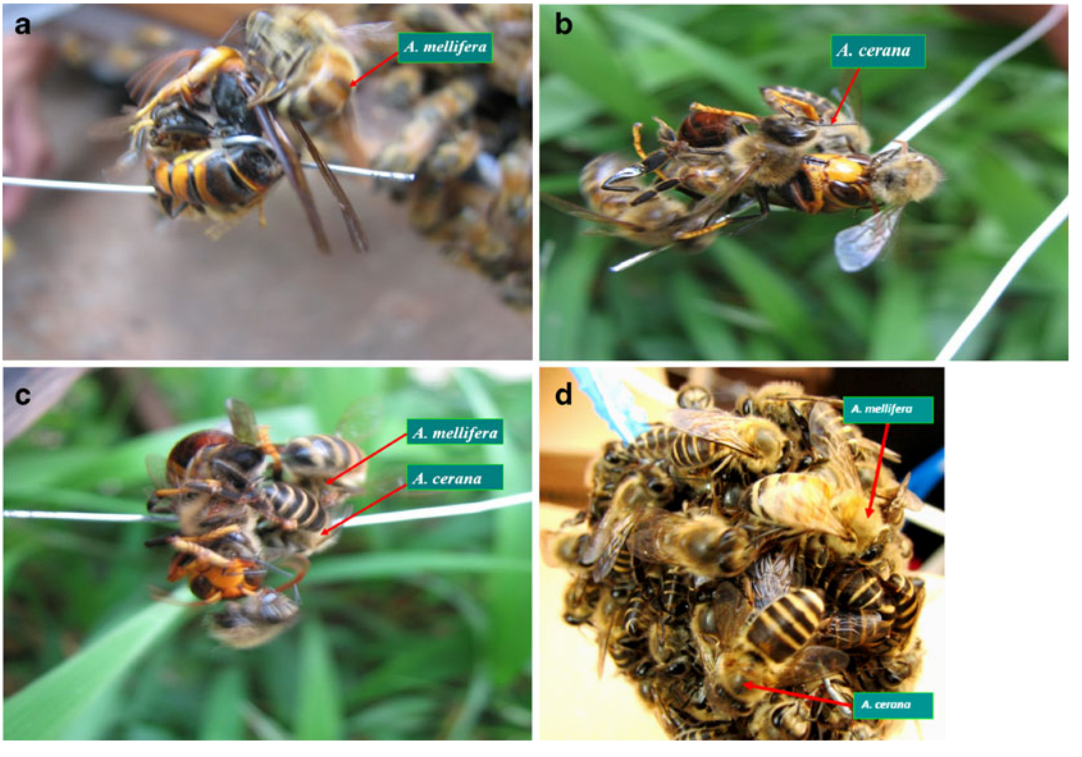

Figure 1. Heat-balling a wasp, *Vespa velutina*, (centre) by a mixed-species colony of *Apis cerana* and *Apis mellifera*. **a** an *A. mellifera* worker begins to fight with wasp and was bitten by it; **b** more *A. mellifera* and *A. cerana* join the fray; **c** even more *A. mellifera* and *A. cerana* join the balling of the wasp; **d** Mixed heat-balling by *A. mellifera* and *A. cerana*.

losses proportionally than *A. mellifera*. But, it must be clearly understood that here we used a highly inbred strain of commercial and docile Chinese honeybee stock. Given the well-known aggressiveness in African races, particularly *A. m. scutellata* (Hepburn and Radloff 1998), we would expect the *A. cerana*/*A. mellifera* differences reported here to be reversed.

Task allocation in honeybees is very complicated probably because there is no central information source available and no "controller" bees engaged in task allocation. It has been suggested that each individual has to make its own decisions which could be produced as a self-organization mechanism (Bonabeau et al. 1997; Page and Mitchell 1998; Kastberger et al. 2011). This mechanism is sufficiently effective that each work force is properly "arranged". Thus, if one task is being done by enough nest mates, newly recruited individuals might stop to perform other tasks, and this might explain the phenomena of *A. cerana* and *A. mellifera* workers heat-balling performances in our mixed colonies.

ACKNOWLEDGEMENTS

Financial support was granted to Ken Tan by Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, China; and to Ming-Xian Yang by Rhodes University, South Africa, and Yunnan Agricultural University, China.

Coopération dans l'élimination des guêpes chez les colonies mixtes d'abeilles, *Apis cerana* et *Apis mellifera*.

coopération / comportement défensif / espèces mixtes / ennemi de la ruche / *Vespa velutina*

Kooperatives Wespentötungsverhalten in Mischkolonien aus *Apis cerana* und *Apis mellifera*

Kooperation / Verteidigungsverhalten / Mischkolonien / *Apis cerana* / *Apis mellifera*

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