Naturwissenschaften (2005) 92: 492–495
DOI 10.1007/s00114-005-0026-5

SHORT COMMUNICATION

Tan Ken · H. R. Hepburn · S. E. Radloff · Yu Yusheng · Liu Yiqiu · Zhou Danyin · P. Neumann

Heat-balling wasps by honeybees

Received: 14 June 2005 / Accepted: 3 August 2005 / Published online: 8 September 2005

Abstract Defensiveness of honeybee colonies of *Apis cerana* and *Apis mellifera* (actively balling the wasps but reduction of foraging) against predatory wasps, *Vespa velutina*, and false wasps was assessed. There were significantly more worker bees in balls of the former than latter. Core temperatures in a ball around a live wasp of *A. cerana* were significantly higher than those of *A. mellifera*, and also significantly more when exposed to false wasps. Core temperatures of bee balls exposed to false wasps were significantly lower than those exposed to *V. velutina* for both *A. cerana* and for *A. mellifera*. The lethal thermal limits for *V. velutina*, *A. cerana* and *A. mellifera* were significantly different, so that both species of honeybees have a thermal safety factor in heat-killing such wasp predators. During wasps attacks at the hives measured at 3, 6 and 12 min, the numbers of *Apis cerana cerana* and *Apis cerana indica* bees continuing to forage were significantly reduced with increased wasp attack time. Tropical lowland *A. c. indica* reduced foraging rates significantly more than the highland *A. c. cerana* bees; but, there was no significant effect on foraging by *A. mellifera*. The latency to recovery of honeybee foraging was significantly greater the longer the duration of wasp attacks. The results show remarkable thermal fine-tuning in a co-evolving predator–prey relationship.

Keywords *A. cerana* · *A. mellifera* · Foraging · Defense · Balling temperature · Hornet · *Vespa velutina*

T. Ken · H. R. Hepburn (✉) · L. Yiqiu · Z. Danyin
Xishuangbanna Tropical Botanical Garden, Chinese Academy of Science Eastern Bee Research Institute of Yunnan Agricultural University,
Heilongtan, Kunming, Yunnan Province, China
e-mail: r.hepburn@ru.ac.za

H. R. Hepburn · P. Neumann
Department of Zoology and Entomology, Rhodes University,
Grahamstown 6140, South Africa

S. E. Radloff
Department of Statistics, Rhodes University,
Grahamstown 6140, South Africa

Y. Yusheng
Bee Research Institute of Yunnan Agricultural Academy of Science Mongzi, Yunnan Province, China

P. Neumann
Institut für Zoologie, Molekulare Ökologie,
Martin-Luther-Universität Halle-Wittenberg,
Hoher Weg 4,
06099 Halle, Saale, Germany

Introduction

When worker honeybees ball another bee the temperature increases in the core of the bee ball (Esch 1960), as it also does in individual worker bees attacking predators (Heinrich 1979) and in guard bees of *A. mellifera* (Stabentheiner et al. 2002). Defensive bee balling is particularly pronounced in the Asian honeybees, *A. cerana* (Ono et al. 1987) in response to wasp attacks (Matsuura and Sakagami 1973; Ken Tan and Wang 2004). When a hornet persists in attack, several worker bees engulf it in a ball and kill it by raising the core temperature "heat-balling" the wasps (Matsuura and Sakagami 1973; Ono et al. 1987). While 20–30% of a colony of *A. cerana* may succumb to the predations of the wasp *V. velutina*, losses are even greater in the introduced European honeybee, *A. mellifera* (Sakagami 1960; Liang Qun 2001). We performed experiments on the defensiveness of two species of honeybees against *V. velutina* to (1) further develop a comparative ethology of these interactions, (2) to assess inter- and intraspecific differences in honeybees and (3) to document the thermal characteristics of such encounters.

Materials and methods

Test colonies

For the temperature balling experiment six colonies each of *A. cerana* and *A. mellifera* and of equal size (four combs of bees) were used. For the foraging assays, colonies were obtained from the southern tropical lowlands (*A. cerana*

indica, *N*=3 colonies) and from the temperate north of Yunnan (*A. cerana cerana*, *N*=3 colonies each for both wasps and false wasps) and *A. mellifera* colonies (*N*=3 colonies) from the apiaries of the Eastern Bee Research Institute of Yunnan Agricultural University and the Bee Institute of Yunnan Agricultural Academy of Science, Yunnan, China (Tables 1 and 2).

Balling temperatures and lethal limits experiments

Wasps and false wasps were glued to the tips of a 2 mm diameter copper wire, which transmitted the temperature to a semiconductor thermal device (Sensortek Company, USA). When a wasp was placed ~20 cm in front of the hive, worker bees engulfed it in a ball in less than 1 s and the number of workers was recorded. The temperature inside the ball was recorded every 15 s for 5 min. The number of workers in a ball and the temperatures inside the ball were recorded for *A. cerana* and *A. mellifera* colonies (*N*=3, with wasps and *N*=3, false wasps, each).

To determine the thermal lethal limits of the honeybees and wasps, *A. cerana* (*N*=10) and *A. mellifera* (*N*=10) and wasps (*N*=10) were placed in three separate cages (10×10×8 cm) in an incubator. The initial temperature of 42°C was raised 1°C every 20 min until 52°C. The number of dead bees and wasps were noted for each degree increase in temperature.

Honeybee foraging and exposure to wasps

Adult wasps were fixed at the end of sticks and placed 20 cm in front of *A. c. cerana* (*N*=3 colonies), *A. c. indica* (*N*=3

Table 1 Means and standard errors of foraging numbers with wasp interfering times (*N*=3 colonies for each trial)

Disturbance time (min)	*A. c. indica*	*A. c. cerana V. velutina*	*A. mellifera*	*A. c. cerana* False wasp
0	39.00±2.52[a]	38.33±1.20[a]	65.00±1.15	34.00±0.58
3	14.33±0.33*	16.67±0.33*	58.33±2.19	35.33±1.20
6	3.67±0.33*	6.00±0.58*	60.33±3.84	35.00±1.53
12	0.33±0.33*	3.33±0.33*	62.67±3.33	34.67±0.33

*Significant intraspecific differences with initial foraging levels (Bonferroni adjustment of α=0.0167)

[a]No significant interspecific difference in initial foraging numbers between *A. c. indica* and *A. c. cerana*

Table 2 Means and standard errors of recovering forage numbers for *A. c. indica*, *A. c. cerana* and *A. mellifera* recorded every minute after withdrawal of the wasp or false wasp (*N*=3 colonies for each trial)

Disturb	Time (min) Recovery	A. c. indica	A. c. cerana *V. velutina*	A. mellifera	A. c. cerana False wasp
3	Initial no	39.00±2.52	38.33±1.20	65.00±1.15	34.00±0.58
	1	29.00±1.00*	28.33±0.33*	59.67±3.48	39.67±2.60
	2	28.67±1.45*	32.33±1.20*	59.00±3.51	37.67±3.84
	3	32.67±0.67	33.33±1.45*	55.67±0.88	35.67±1.20
	4	36.67±0.67	33.33±2.85	59.33±3.18	37.33±1.86
	5	39.67±2.03	33.33±0.88	59.00±1.16	34.67±0.33
6	Initial no	32.67±0.88	31.33±0.88	65.00±2.08	41.00±0.58
	1	18.00±1.00*	14.33±0.88*	61.00±2.65	38.00±2.08
	2	18.33±0.88*	16.67±0.88*	52.67±2.03	35.33±1.20
	3	23.67±1.20*	17.67±0.67*	60.33±2.96	34.33±3.53
	4	24.67±1.20*	20.33±0.88*	59.33±2.19	38.00±1.53
	5	28.33±0.67*	23.67±0.88*	62.67±3.53	36.00±2.65
	6	32.33±2.85	23.33±1.20*	62.67±2.33	39.33±2.40
12	Initial no	25.67±0.88	32.67±0.88	57.33±5.33	37.33±1.45
	1	1.00±0.58*	3.33±0.88*	63.00±5.57	34.67±0.88
	2	4.67±2.03*	7.33±1.20*	60.00±5.29	33.67±1.76
	3	5.00±0.58*	13.00±1.16*	59.67±3.67	36.00±1.53
	4	11.00±1.16*	15.00±0.58*	59.67±3.75	35.00±1.53
	5	12.33±0.88*	15.67±0.88*	55.33±3.48	38.67±1 67
	6	19.00±0.58*	15.00±1.00*	56.33±2.33	36.67±2.19
	7	24.33±0.88	17.00±1.73*	57.33± 3.84	37.00±2.31
	8	24.67±1.45	17.33±1.20*	65.67± 0.67	35.33±1.45
	9	27.67±1.76	21.00±2.08*	66.00±0.58	33.33±2.19
	10	28.33±0.67	26.67±0.88*	63.00±3.51	34.33±1.20
	11	30.67±1.86	29.33±0.67	62.33± 4.18	35.33±4.33
	12	32.33±2.96	30.00±0.58	56.33±1.45	37.00±4.04

*Significant intraspecific difference compared to initial number of foragers (Bonferroni adjustment of α=0.0167)

colonies) and *A. mellifera* (*N*=3 colonies) hive entrances and held there for 3, 6 and 12 min (simulating their hawking patrols). *A. c. cerana* (*N*=3 colonies) were also exposed to false wasps. The numbers of foraging workers were counted every minute. Tests were repeated five times for both wasps and false wasps. The recovery times (in min) to initial foraging levels were also recorded. The false wasps were made of plastic and painted to mimic *V. velutina*.

Data analysis

Independent samples *t*-tests were used for testing interspecific ball temperature differences between *A. cerana* and *A. mellifera* and temperature differences between balls exposed to *V. velutina* and false wasps. ANOVA and Scheffe multiple comparison procedures were used to test for lethal thermal limits differences between *V. velutina*, *A. cerana* and *A. mellifera*. Matched *t*-tests were used to test for intraspecific differences in the number of foragers at intruder interfering times with initial foraging levels. Homogeneity of the variances between groups was checked using Levene's test.

Results

Temperature changes in bee balls

When adult wasps or false wasps were put at the entrance of an *A. cerana* hive, guard bees engulfed them in balls of 32.2±3.2 workers the inner temperature of which increased sharply to 44.6±0.1°C for false wasps and 45.3±0.1°C for live wasps in 210 s, finally reaching 44.9±0.1°C and 45.6±0.1°C, respectively at the end of 5 min. At the *A. mellifera* hives, 22.7±3.1 workers produced a ball temperature of 42.2±0.2°C for false wasps and 44.1±0.1°C for live *V. velutina* in 210 s and, finally 43.1±0.1°C and 44.3±0.2°C, respectively in 5 min (Fig. 1). The mean number of worker bees in *A. cerana* balls was significantly higher than that of *A. mellifera* (*t*-tests: t=5.25, 10 df, p=0.0004).

There were no significant differences in initial temperatures between *A. cerana* balls exposed to live and false wasps after 15 s (*t*-test, t=2.00, 4 df, p=0.1161); nor between *A. mellifera* exposed to live and false wasps after 15 s (*t*-test, t=0.90, 4 df, p=0.4167). There were no interspecific differences in initial temperatures between *A. cerana* and *A. mellifera* exposed to live and false wasps (*V. velutina*: *t*-test, t=0.63, 4 df, p=0.5614; false wasps: *t*-test, t=1.70, 4 df, p=0.1648); but, there were indeed differences in temperatures recorded after 5 min between *A. cerana* and *A. mellifera* balls for both live and false wasps (*V. velutina*: *t*-test: t=13.8, 4 df, p=0.0002; false wasps: t=38.2, 4 df, p<0.0001). Temperatures of *A. cerana* balls were significantly higher than those of *A. mellifera* by 1.3 and 1.8°C when exposed to live and false wasps, respectively.

Significant differences in temperatures recorded at 5 min were also found between live and false wasps for both species of honeybees (*A. cerana*: *t*-test: t=13.4, 4 df, p=0.0002; *A. mellifera*: t=12.0, 4 df, p=0.0003). Inner temperatures of balls when bees were exposed to false wasps were significantly lower than those exposed to *V. velutina* by 0.7°C for *A. cerana* and 1.2°C for *A. mellifera*.

Thermal lethal limits of A. cerana, A. mellifera and V. velutina

When the temperature reached 45°C, three wasps were dead and the remaining seven at 46°C, but all 20 honeybees were still alive. At 51°C, two *A. mellifera* were dead and another eight at 52°C; at 50°C, three *A. cerana* were dead and another seven at 51°C. The lethal thermal limit for *V. velutina* was 45.7±0.48°C, and for *A. cerana* and *A. mellifera* 50.7±0.48°C and 51.8±0.42°C, respectively. These means are significantly different overall (ANOVA: F=492.3, 2,27 df, p<0.0001) and also each paired group comparison of the means is significantly different (Scheffe: p<0.0001).

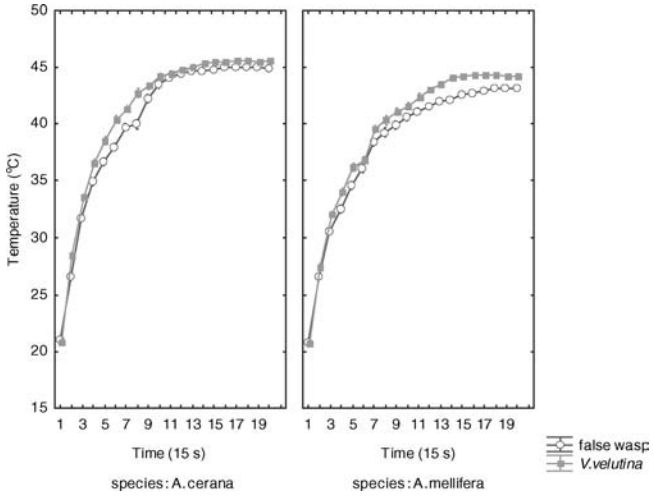

Fig. 1 Graph of mean temperature variation (°C) against time (15 s) inside *A. cerana* and *A. mellifera* balls for live and false wasps (*N*=3)

Variation in foraging numbers

The numbers of foraging *A. c. cerana* and *A. c. indica* were significantly reduced with increasing exposure of the wasps (matched *t*-tests: 3 min: t=16.8, 5 df, P<0.0001; 6 min: t=29.9, 5 df, P<0.0001; 12 min: t=25.9, 5 df, P<0.0001; Table 1). No significant intraspecific differences in the numbers of foraging *A. mellifera* or *A. cerana* were observed when exposed to *V. velutina* or false wasps, respectively (*t*-test: t=0.63, 4 df, p=0.5614).

A. cerana from the tropical lowlands were more sensitive to wasp exposure than those from the temperate highlands (*A. c. indica* slope: β=−0.2892, R^2=0.96, p<0.0001; *A. c. cerana* slope: β=−0.1831, R^2=0.90, p<0.0001; interspecific slope difference test: p=0.0142). Interestingly, *V. velutina* wasp exposure had no significant effect on the foraging levels of *A. mellifera* (slope: β=−0.001, R^2=0.003, p=0.8647) and false wasp exposure had no

apparent effect on the foraging levels of *A. cerana* (slope: β=0.002, R^2=0.017, p=0.6882).

Variation in latency to foraging recovery times

The longer the duration of wasp exposure to *A. cerana*, the longer the recovery time to initial foraging levels. The number of *A. c. indica* foragers returned to initial level sooner than in *A. c. cerana*. When exposure was only 3 min, foraging number was reduced but returned to the initial level after 3 min for *A. c. indica* and 4 min for *A. c. cerana* (no significant difference with initial level: *A. c. indica* after 3 min, matched *t*-test: t=2.02, 4 df, p>0.113; *A. c. cerana* after 4 min, matched *t*-test: t=1.98, 4 df, p>0.199, Table 2). When wasp exposure was increased to 6 min, foraging number declined more obviously, and returned to initial levels after 5–6 min (no significant difference with initial level: *A. c. indica* after 5 min, matched *t*-test: t=0.11, 4 df, p>0.914; *A. c. cerana* after 6 min, matched *t*-test: t=2.79, 4 df, p=0.049, Table 2). Exposed to wasps for 12 min, foraging ceased and only some bees balled the wasp. After the wasp was removed, bees began to fly out in small numbers, and were restored to undisturbed foraging levels after about 7 min for *A. c. indica* and 11 min for *A. c. cerana* (no significant difference with initial level: *A. c. indica* after 7 min, matched *t*-test: t=0.45, 4 df, p>0.678; *A. c. cerana* after 11 min, matched *t*-test: t=2.06, 4 df, p>0.109, Table 2).

Discussion

The aggressive defensive behaviour of *A. cerana* is more efficient than that of *A. mellifera* colonies both in terms of recruited workers and balling temperature increases and, passively in discontinuing foraging when under wasp attack. Worker recruitment averaged 32.2±3.2 in the former and 22.7±3.1 in the latter workers. Due to a lack of co-evolutionary history, one might expect less efficient balling behaviour in a honeybee species such as *A. mellifera*, which is not sympatric with such vespine predators. However, the occurrence of thermal balling in defense could well predate speciation in *Apis* because it also occurs in *A. dorsata* (Kastberger and Stachl 2003), a clade remote from *A. cerana* and *A. mellifera* (Alexander 1991).

The final core temperature of the *A. cerana* balls rose faster and higher (1.3 and 1.8°C) for both live and false wasps than in *A. mellifera* (p<0.05). The maximum inner temperatures of balls for live and false wasps were significantly different (p<0.05) for both *A. cerana* and *A. mellifera* colonies. During balling, high temperatures are generated by rapid increases in the metabolism of honeybee workers through non-shivering thermogenesis as in A. dorsata (Kastberger and Stachl 2003). Our results complement previous data that high temperatures prove lethal to predatory vespid wasps, but not to the bees themselves (Ono et al. 1987).

Foraging of *A. cerana* colonies was significantly reduced in the presence of wasps. Indeed, during the experiments the typical smell of iso-pentyl acetate, a major alarm pheromone component in *Apis*, emanated from the balling bees. This suggests that foraging reduction is induced by olfactory cues, while balling is more likely induced by visual and olfactory stimuli the release of which is amplified by an increased cuticular temperature (Stabentheiner et al. 2002) and possibly also by hissing (Koeniger and Fuchs 1973; Sen Sarma et al. 2002) which could be perceived through the clump of bees as a substrate themselves (Michelsen 1998). That foraging is not reduced in *A. mellifera* accounts for the greater wasp depredations it suffers in Asia.

The tropical lowland *A. c. indica* colonies were more sensitive than the temperate highland *A. c. cerana* to the wasps; and, both were more sensitive than *A. mellifera* colonies. During wasp exposure, the number of lowland foragers decreased faster, and after the wasp left, returned to initial foraging levels sooner than in the highland subspecies. False wasps do not affect foraging activity, probably because they lack the cues necessary to reduce foraging flights. Nevertheless, they are able to induce balling behaviour (Ono et al. 1987). The differences in colony defense among honeybee species indicate that those honeybee species (*cerana*, *dorsata*, *nuluensis*) sympatric with wasps have evolved an especially efficient colony level defence system.

References

Alexander B (1991) A cladistic analysis of the genus *Apis*. In: Smith DR (ed) Diversity in the Genus *Apis*. Westview Press, Boulder

Esch H (1960) Über die Körpertemperaturen und den Wärmehaushalt von *Apis mellifica*. Z Vergl Physiol 43:305–335

Free JB (1987) Pheromones of social bees. Chapman and Hall, London

Heinrich B (1979) Thermoregulation of African and European honeybees during foraging. J Exp Biol 80:217–229

Ken T, Wang JM (2004) Reduction of foraging activity by *A. cerana* colonies attacked by *Vespa velutina*. J Bee 2:7–9

Kastberger G, Stachl R (2003) Infrared imaging technology and biological applications. Behav Res Methods Instrum Comput 35:429–439

Koeniger N, Fuchs S (1973) Sound production as colony defence in *Apis cerana*. Proc Int IUSSI Congr 7:199–204

Qun L (2001) Bee disease and pest control. Apiculture in China, pp 583–658

Matsuura M, Sakagami SF (1973) A bionomic sketch of the giant hornet, *Vespa mandarinia*, a serious pest for Japanese apiculture. J Fac Sci Hokkaido Univ VI 19:125–162

Michelsen A (1998) Biophysics of sound localization in insects. In: Hoy RR, Popper AN, Fay RR (eds) Comparative hearing: insects. Springer Handbook of Auditory Research, p 18–62

Ono M, Okada I, Sasaki M (1987) Heat production by balling in the Japanese honeybee, *Apis cerana japonica* as a defensive behavior against the hornet, *Vespa simillima xanthoptera* (Hymenoptera: Vespidae). Experientia 43:1031–1032

Sakagami SF (1960) Preliminary report on the specific difference behaviour and the other ecological characters between European and Japanese honeybee. Acta Hymenopterol 1:171–198

Sen Sarma M, Fuchs S, Werber C, Tautz J (2002) Worker piping triggers hissing for coordinated colony defence in the dwarf honeybee *Apis florea*. Zoology 105:215–223

Stabentheiner A (1996) Thermische Aggression im Bienenvolk. Verhandlungen der Deutschen Zoologischen Gesellschaft 89.1:297

Stabentheiner A, Kovac H, Schmarranzer S (2002) Honeybee nestmate recognition: The thermal behaviour of guards and their examinees. J Exp Biol 205:2637–2642