

The ‘I see you’ prey–predator signal of *Apis cerana* is innate

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Abstract An ‘I see you’ (ISY) prey–predator signal can co-evolve when such a signal benefits both prey and predator. The prey benefits if, by producing the signal, the predator is likely to break off an attack. The predator benefits if it is informed by the signal that the prey is aware of its presence and can break off what is likely to be an unsuccessful and potentially costly hunt. Because the signal and response co-evolve in two species, the behaviour underlying an ISY signal is expected to have a strong genetic component and cannot be entirely learned. An example of an ISY signal is the ‘shimmering’ behaviour performed by Asian hive bee workers in the presence of their predator *Vespa velutina*. To test the prediction that bee–hornet signalling is heritable, we let honey bee workers of two species emerge in an incubator so that they had never been exposed to *V. velutina*. In *Apis cerana*, the shimmering response developed 48 h post-emergence, was strong after 72 h and increased further over 2 weeks. In contrast, *A. mellifera*, which has evolved in the absence of Asian hornets, did not produce the shimmering signal. In control tests, *A. cerana* workers exposed to a non-threatening butterfly did not respond with the shimmering signal.

Keywords Pursuit deterrent signalling · Prey–predator signalling · *Apis cerana* · *Apis mellifera* · *Vespa velutina* · Species recognition

Introduction

‘I see you’ (ISY) or ‘prey–predator’ signals occasionally co-evolve between a prey and a predator species (Walther 1969; Caro 1986; Ruxton et al. 2004). An ISY signal (analogous to a pursuit deterrent signal in vertebrates) informs a predator that it has been seen, and that further approach is likely to be futile because it will be outrun, mobbed, or otherwise prevented from making a kill (Vega-Renondo and Hasson 1993; Bergstrom and Lachmann 2001). The prey species benefits from its ISY signal because attacks by predators are reduced. The predator species benefits because it can break off attacks that are unlikely to succeed and may be dangerous without the element of surprise. Because ISY signals evolve by natural selection, production of the ISY should have a genetic component, released by the presence of the predator. Similarly, the response of the predator—breaking off the attack when the prey produces the signal—should also have a genetic component.

We recently suggested that the shimmering behaviour of the Asian hive bee *Apis cerana* in the presence of its predator the hornet *Vespa velutina* is an example of an ISY signal (Tan et al. 2012). When a hornet approaches a colony, workers gather at the nest entrance and vigorously shake their abdomens laterally in unison, producing a loud buzzing noise, and a group characteristic shimmering display (Sakagami 1960; Butler 1974; Koeniger and Fuchs 1975; Oldroyd and Wongsiri 2006; Tan et al. 2007). We suggested that *A. cerana* workers signal approaching hornets that they have been detected and will be killed if they approach the hive entrance (Tan et al. 2012). *V. velutina* responds to the

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bee's signal by moving away from the nest entrance, where it will usually attempt (mostly unsuccessfully) to hunt bees in the air as they approach the colony (Kloft and Schneider 1969; Abrol 2006; Fuchs and Tautz 2011). The bee's signal is 'honest' in that should the hornet land on the nest entrance, it is 'balled' by up to 150 defending bees that pack around the intruder and kill it with heat and suffocation (Ono et al. 1987; Ono et al. 1995; Ken et al. 2005). Thus, the bees can back up their ISY display with lethal force. In contrast, a sibling honey bee species, the western hive bee *Apis mellifera*, which did not evolve in the presence of Asian hornets, does not produce the shimmering signal and is incapable of defending by heat balling (Ono et al. 1995; Ken et al. 2005). Examples of ISY signals are controversial (Caro 1995; but see Clark 2005; Blumstein 2007; Rundus et al. 2007; Rao and Diaz-Fleischer 2012 for recent examples), and so the honey bee–hornet case is of interest as an experimental system for exploring the evolution of inter-specific signalling (Sendova-Franks 2012; Tan et al. 2012).

A behaviour may be described 'innate' if it is executed 'on the first available opportunity to occur, without the possibility of previous learning' (Verplanck 1955). Here, we determine whether the shaking signal of *A. cerana* workers in response to the presence of a hornet is indeed inherited by exposing naïve workers of both *A. cerana* and *A. mellifera* to tethered hornets and non-threatening butterflies. If naïve *A. cerana* workers produce the shimmering signal only in the presence of hornets and not in the presence of butterflies, and *A. mellifera* workers do not produce the signal under any circumstances, then this would provide further support for the hypothesis that shimmering is indeed a co-evolved ISY signal because it is a species-specific inherited behaviour.

Materials and methods

We observed the behaviour of naïve workers from three colonies each of *A. cerana* and *A. mellifera* in response to their predator *V. velutina* and a benign butterfly (*Papilio xuthus*). Observations were carried out from May to August of 2012 at Yunnan Agricultural University.

To set up experimental 'colonies' (three per species), we caged the queen on an empty comb to lay eggs, thus generating a comb in which all brood would emerge within 12 h. The day before the workers were due to emerge, we placed the comb in an empty nucleus hive, and then placed the hive in an incubator at 33 °C. The next day, after the workers had emerged, we transferred an incipient colony to a bee flight room (4.5×5.5×3 m) in which the ambient temperature was 21–27 °C. We fed the bees with sucrose syrup ad libitum until the conclusion of the experiment, and stored pollen was available in the brood combs. We did not introduce a queen.

To obtain target insects with which to observe the bee's reaction, we caught hornets and butterflies in insect nets in and around the apiary. Thus, the hornets were likely to be experienced bee hunters. We affixed each live insect to a piece of wire (2.2 mm diameter and 50 cm long) by tying it by the waist to the holder wire while the insect was restrained in the net. Target insects were of similar size: *V. velutina* has a head–tail length of about 21 mm and *P. xuthus* 25 mm.

Without smoke, we then removed the comb and its adhering workers from the experimental hive in the flight room and exposed the workers to the tethered hornet or butterfly within the flight room. We waved the target insect from side-to-side through an arc of about 20 cm at a speed of about 20 cm/s, 5 cm from one side of the comb for 1 min. (This movement is typical of *V. velutina* when it hunts in front of *A. cerana* colonies, personal observations of K. Tan.) To record the bee's behavioural responses, we placed a digital video camera (Panasonic NV-GS400GC) about 20 cm in front of the comb. We recorded the behaviour of the workers at a rate of 25 images per second, which is sufficiently frequent to capture all of the typically 0.3 s bouts of shimmering. Observations were conducted in the afternoon when the ambient temperature was around 25 °C.

For each colony, we first presented a tethered hornet and followed immediately by a tethered butterfly. The comb was returned to the hive for 30 min and the test was repeated with the butterfly presented first. The comb was again returned to the hive for 30 min and the hornet presented again, immediately followed by the butterfly (i.e., three tests were performed 30 min apart on the same day). From the videos, we counted the total number of bees in the field of view at the time of introduction of the test insect, and the total number of bees that performed the shaking movement at any time during the 1-min test. We also selected 10 individual *A. cerana* workers at the beginning of each recording and recorded the total number of bouts of shaking for each of the 10 workers in the 1-min test of each trial.

To determine if there was any difference in the behaviour of the bees as they matured from the age at which they would typically nurse brood (0–72 h) to the age at which they typically begin guarding (14 days), we repeated the above trial when bees were 24, 48 and 72 h old and then again when they were 14 days old.

For statistical analysis of the proportion of bees that performed the shaking movement, we averaged the three pseudo-replicate trials for each colony on each day and performed repeated-measures ANOVA with bee age a fixed, repeated effect and threat (butterfly or hornet) the main effect. Prior to ANOVA, we transformed the proportional data with a $\log_{10}(x+1)$ transformation, but we present untransformed means in the figures. We used the Greenhouse–Geisser degrees of freedom adjustment for sphericity.

For the analysis of the number of shakes per minute per bee, we transformed the data with a $\sqrt{X + 1/2}$ transformation but report untransformed means in the figures. We regarded the 10 bees as individual replicates, and analysed age as a repeated measure and colony as a fixed effect.

Results

We scored the behaviour from 2,100 observations of *A. cerana* and 3,686 observations of *A. mellifera* bees, all from three colonies of each species. An average of 58.3 ± 6.4 (s.d.) *A. cerana* and 102.4 ± 11.8 *A. mellifera* workers were in frame per recording. Because of the finite number of workers in the test colonies, estimated to be 400–800 workers, the behaviour of the same bees was recorded repeatedly over the course of the experiment.

All naïve *A. cerana* colonies shimmered in response to hornet predators when workers were one or more days of age. There was a significant increase ($F_{2,02, 8.11} = 502.8$, $P < 0.001$) in the proportion of *A. cerana* workers that produced the shaking movement in the presence of a hornet as the workers aged, such that at 2 weeks of age $64.4 \pm \text{s.e. } 2.0$ % of workers shook in the presence of a hornet (Fig. 1). No workers produced the shaking movement in the presence of the butterfly until the bees were 2 weeks old (Fig. 1). Even at 2 weeks of age, only 1.3 ± 2.0 % of workers produced the shaking movement in the presence of a butterfly. Overall, the threat species had a highly significant effect on the proportion of *A. cerana* bees that performed the shaking movement ($F_{1,4} = 3,468.8$, $P < 0.001$).

Not only did the proportion of *A. cerana* workers that perform the shaking movement increase with age but the number of bouts of shaking per minute similarly increased

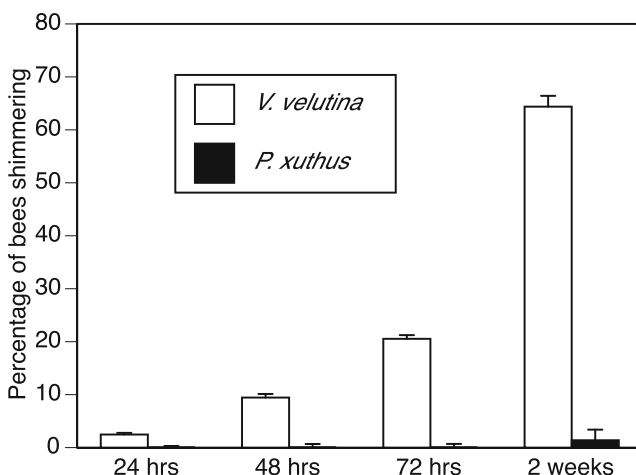


Fig. 1 Proportion of *A. cerana* workers performing the lateral shimming movement in the presence of *V. velutina* or *P. xuthus*. Error bars are standard error of the means

with age ($F_{1,62,216} = 182.14$, $P < 0.001$, Fig. 2). The effect of colony was not significant over all ages ($F_{2, 54} = 0.13$, $P = 0.88$), and the effect of the threat (butterfly or hornet) was highly significant ($F_{1,54} = 733.67$, $P < 0.001$).

No *A. mellifera* workers produced the shaking movement in the presence of a butterfly or hornet at any age (data not shown).

Discussion

Young *A. cerana* workers emerged in isolation from older siblings can perform the shimmering display without prior experience of hornets. Workers do not need to learn that *V. velutina* is an enemy by witnessing hunting events. They distinguish hornets from similarly sized butterflies. *A. cerana* almost never produces the shimmering display in the presence of butterflies (which are larger and more conspicuous than *V. velutina*), even though we moved the butterfly in a way that mimicked the hunting movements of the hornet. This is strong evidence that the shaking behaviour is innate and tuned to the presence of the bee's major predator group, Asian hornets. That the behaviour is genetically determined and tuned to a specific predator that responds to the signal (Tan et al. 2012) provides further evidence that the shimmering of Asian hive bees is an ISY signal that has co-evolved between hornet and bee.

A. mellifera workers do not perform the behaviour, and they have not co-evolved with Asian hornets. It could be argued that under more natural experimental conditions, *A. mellifera* workers might perform the shaking movement. However as *A. mellifera* have never been seen to produce the display in vivo, we do not believe that the artificial conditions of our experiment caused the absence of the behaviour. Rather, the signal is absent from the species.

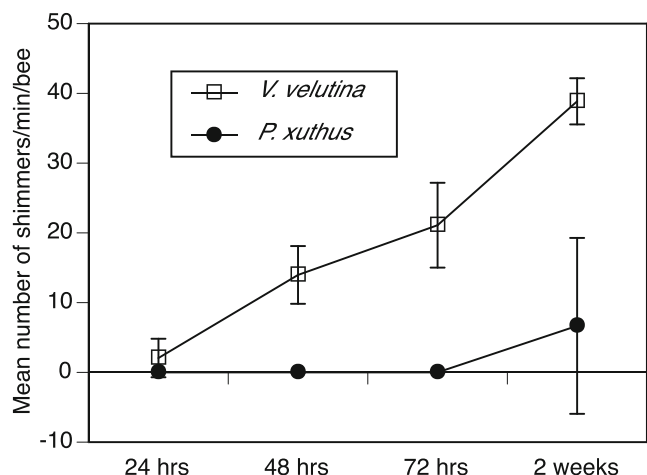


Fig. 2 Mean number of shaking movements per minute performed by 10 random *A. cerana* workers in the presence of *V. velutina* or *P. xuthus*. Error bars are standard deviations

The proportion of *A. cerana* bees that engage in the shimmering display increases with the bee's age, so that the majority of 2-week-old workers engage in it. The number of bouts of shaking per 1 min test also increases with age. This may suggest that the behaviour increases in frequency as the bees enter the period when they commence guarding duties. This assumption is speculative because age polyethism, although well described in *A. mellifera* (Rösch 1925; Seeley and Kolmes 1991), is not well documented for *A. cerana*. We also caution that while age is the most likely reason why the frequency of shimmering increases, we cannot discount the possibility that experience may also have played a role.

The Asian honey bee/hornet interaction provides an example of an ISY signal that can be experimentally manipulated. The behaviour is innate in the bee, indicating that it has arisen through natural selection because it successfully deters predators. The predator responds to the signal indicating that the cost of not responding appropriately, death by heat balling, has selected for avoidance behaviour by the wasp whenever the bees signal. Compelling examples of ISY signals are rare in nature. In many reported instances of prey–predator signals, the human observer is the surrogate for the predator (Hasson 1991). In these systems, the natural behaviour of the predator cannot be investigated, and this in turn may affect the behaviour of the prey (Caro 1995). In the *A. cerana/V. velutina* interaction, we have a system where both predator and prey can be experimentally manipulated to help reveal how these signals evolve.

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References

- Abrol DP (2006) Defensive behaviour of *Apis cerana* F. against predatory wasps. *J Apic Sci* 50:39–46
- Bergstrom CT, Lachmann M (2001) Alarm calls as costly signals of antipredatory vigilance: the watchful babbler game. *Anim Behav* 61:535–543
- Blumstein DT (2007) Feeling the heat: ground squirrels heat their tails to discourage rattlesnake attack. *Proc Nat Acad Sci U S A* 104:14177–14178
- Butler CG (1974) The world of the honeybee. Collins, London
- Caro TM (1986) The functions of stotting in Thompson's gazelles: some tests of the predictions. *Anim Behav* 34:663–684
- Caro TM (1995) Pursuit-deterrence revisited. *Trends Ecol Evol* 10:500–503
- Clark RW (2005) Pursuit-deterrent communication between prey animals and timber rattlesnakes (*Crotalus horridus*): the response of snakes to harassment displays. *Behav Ecol Sociobiol* 59:258–261
- Fuchs S, Tautz J (2011) Colony defence and natural enemies. In: Hepburn HR, Radloff SE (eds) *Honeybees of Asia*. Springer, Heidelberg, pp 369–395
- Hasson O (1991) Pursuit-deterrent signals: communication between prey and predator. *Trends Ecol Evol* 6:325–329
- Ken T, Hepburn HR, Radloff SE, Yusheng Y, Yiqiu L, Danyin Z, Neumann P (2005) Heat-balling wasps by honeybees. *Naturwissenschaften* 92:492–495
- Kloft W, Schneider P (1969) Gruppenverteidigungsverhalten bei wildlebenden Bienen (*Apis cerana* Fabr.) in Afghanistan. *Naturwissenschaften* 56:219
- Koeniger N, Fuchs S (1975) Zur Kolonieverteidigung der asiatische Honigbienen. *Zeitschrift für Tierpsychologie* 37:99–106
- Oldroyd BP, Wongsiri S (2006) Asian honey bees. Biology, conservation and human interactions. Harvard University Press, Cambridge
- 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
- Ono M, Igarashi T, Ohno E, Sasaki M (1995) Unusual thermal defence by a honeybee against a mass attack by hornets. *Nature* 377:334–336
- Rao D, Diaz-Fleischer F (2012) Characterization of predator-directed displays in Tephritid flies. *Ethology* 118:1165–1172
- Rösch GA (1925) Untersuchungen über die Arbeitsteilung im Bienenstaat. I Die Tätigkeiten im normalen Bienenstaat und ihre Beziehungen zum Alter der Arbeitsbienen. *Z Vergl Physiol* 2:571–631
- Rundus AS, Owings DH, Joshi SS, Chinn E, Giannini N (2007) Ground squirrels use an infrared signal to deter rattlesnake predation. *Proc Nat Acad Sci U S A* 104:14372–14376
- Ruxton GD, Sherratt TN, Speed MP (2004) Avoiding attack: the evolutionary ecology of crypsis, warning signals and mimicry. Oxford University Press, Oxford
- Sakagami SF (1960) Preliminary report on the specific differences in behaviour and other ecological characters between European and Japanese honey bees. *Acta Hymenopterologica* 1:171–198
- Seeley TD, Kolmes SA (1991) Age polyethisms for hive duties in honey bees—illusion or reality? *Ethology* 87:284–297
- Sendova-Franks A (2012) Hornet's response to honeybee signal. *Anim Behav* 83:869–870
- Tan K, Radloff SE, Li JJ, Hepburn HR, Yang MX, Zhang LJ, Neumann P (2007) Bee-hawking by the wasp, *Vespa velutina*, on the honey bees *A. cerana* and *A. mellifera*. *Naturwissenschaften* 94:469–472
- Tan K, Wang Z, Li H, Yan S, Hu Z, Kastberger G, Oldroyd BP (2012) An 'I see you' prey–predator signal between the Asian honeybee (*Apis cerana*) and the hornet (*Vespa velutina*). *Anim Behav* 83:879–882
- Vega-Renondo F, Hasson O (1993) A game-theoretic model of predator–prey signalling. *J Theor Biol* 162:309–319
- Verplanck WS (1955) Since learned behavior is innate and vice versa, what now? *Psych Rev* 62:140–144
- Walther FR (1969) Flight behaviour and avoidance of predators in Thompson's gazelle (*Gazella thomsoni* Guenther 1884). *Behaviour* 34:184–221