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An 'I see you' prey–predator signal between the Asian honeybee, *Apis cerana*, and the hornet, *Vespa velutina*

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When a prey animal displays to a predator, the prey benefits because it is less likely to be attacked, and the predator benefits because it can break off an attack that is unlikely to succeed because the prey has been alerted. We argue that an 'I see you' signal has coevolved between the Asian hive bee, *Apis cerana*, and its hornet predator, *Vespa velutina*. When a hornet approaches a bee colony, guards perform a shaking movement that repels the hornet. To test whether this is an 'I see you' display, we exposed colonies to free-flying and tethered hornets and tethered butterflies. The intensity of the shaking was correlated with the hornet's proximity, whereas guard bees barely responded to a nonthreatening butterfly. The signal is likely to be honest, because the bees can kill the hornet by collective mobbing if it lands on the entrance. The Western honeybee, *Apis mellifera*, which has not evolved in the presence of Asian hornets, does not produce the signal and is ineffective at killing hornets by collective mobbing. We also found that hornets were more successful at catching *A. mellifera* than *A. cerana* bees at the hive entrance.

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To be evolutionarily stable a biological signal must usually be 'honest', and benefit both the receiver and the sender. Calls and displays elicited by predators from their prey species may evolve to inform an approaching predator that it has been detected (Smythe 1970, 1977; Woodland et al. 1980; Hasson 1991). 'I see you' displays (hereafter ISY displays) benefit the prey because they reduce the probability that the predator will escalate the approach to full attack. They also benefit the predator, which can seek alternative prey that has not been alerted (Hasson 1991; Ruxton et al. 2004). ISY displays have been described in some ungulates (Smythe 1970; Hirth & McCullough 1977), where the 'stotting' gait is a signal to the predator that it has been seen and can be outrun (Walther 1969; Caro 1986; FitzGibbon & Fanshawe 1988). Further examples are reviewed in Ruxton et al. (2004). However, we are unaware of reports of ISY displays between insects.

In order for an ISY display to evolve, the predator species must usually be thwarted whenever it attempts to attack after experiencing the ISY display from the prey species (Vega-Renondo & Hasson 1993; Bergstrom & Lachmann 2001). That is, the prey species must be able to back up its ISY with action, by outrunning the predator, collective mobbing, escape to a refuge, or any other mechanism that prevents the predator from making a kill. Without such action, even if it is rarely applied, it is difficult to see how an ISY display could evolve.

The Asian hive bee, *Apis cerana*, appears to have evolved an ISY display (or more appropriately a 'we see you' display since the display is performed by multiple workers simultaneously), showing the characteristics of a signal of recognition of a predator's presence that can be backed up by a defensive action if required. When a potential flying predator, particularly a hornet, approaches an *A. cerana* colony, guard bees at the entrance produce a characteristic shaking signal, in which all the guards simultaneously vibrate their abdomens from side to side for a few seconds (Sakagami 1960; e.g. Butler 1974; Koenig & Fuchs 1975; Oldroyd & Wongsiri 2006).

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The vibration is visually striking (see [Supplementary video](#)), and is accompanied by a loud buzzing noise.

Apis cerana guards are more than capable of backing up their ISY display with defensive action. If a hornet lands at the hive entrance despite the ISY display, it is pounced on by the *A. cerana* guards, which then form a dense ball of up to 500 bees around the hornet (Ono et al. 1995; Tan et al. 2010). The ball of bees kills the hornet with a combination of heat and suffocation (Ono et al. 1987, 1995; Ken et al. 2005). In contrast, the Western hive bee *A. mellifera*, which has not evolved in the presence of Asian hornets, does not produce the shaking display, and is much less effective at heat balling hornets (Ken et al. 2005). Instead, *A. mellifera* guards tend to approach hornets as individuals, and are thus more vulnerable to hornet predation than *A. cerana* guards.

In this study we tested some predictions of the ISY display hypothesis using *A. cerana*, *A. mellifera*, *Vespa velutina* and a nonthreatening butterfly species, *Papilio xuthus*. Colonies of the two honeybee species were exposed to free-flying and tethered hornets and to tethered butterflies. If the shaking display is indeed an ISY display moulded by natural selection to inform hornets that they have been detected and will be heat balled if they approach, then we would expect the following. (1) The intensity of the display should increase with the proximity of the threat (Helfman 1989). In this case the guard's shaking display should increase with proximity of the hornet to the nest. (2) Hornets should be repelled by the display. (3) The display should decrease bee predation. (4) *Apis cerana* guards should not respond with the shaking signal when approached by a nonthreatening species such as a butterfly. Furthermore, hornets should be more likely to approach *A. mellifera* colonies, which cannot produce the shaking display, than *A. cerana* colonies, and be more successful at catching *A. mellifera* foragers and guards than *A. cerana* foragers and guards.

METHODS

Free-flying Hornets

Observations were made in the autumns of 2009 and 2010 at Yunnan Agricultural University, Kunming, China. We observed the behaviour of 10 colonies each of *A. cerana* and *A. mellifera* in response to a hornet attack. Colonies comprised four frames of bees and brood. Observations were conducted in the afternoon when the ambient temperature was about 25 °C. First, we counted the guard bees at the entrance of each colony at the same time on each of 7 days. Second, for each test colony we placed a digital video camera (Panasonic NV-GS400GC) 30–100 cm to the side of a hive entrance. Recordings were made for 1 h per day until we had observed 10 or more hornet visits. To calibrate distances of flying hornets from hive entrances, we placed a ruler within the view of the cameras. We selected 10 hornet visits per colony by randomly choosing 10 digital recordings and measured: (1) the distance from the nest entrance at which the hornet first appeared; (2) the median distance of hovering hornets to the entrance; (3) the hornet's hovering time; and (4) the number of bees captured.

Tethered Hornets and Butterflies

To determine whether *A. cerana* guard bees' shaking response is correlated with the proximity and flight speed of hovering hornets and nonthreatening butterflies, we tethered live hornets and live butterflies, somewhat larger than the hornets, with conspicuous yellow and black warning coloration, to pieces of wire (1.0 mm diameter), in the autumn of 2011. For each of three trials for 10 colonies of each species we then recorded (at a rate of 25 images/s) the responses of the guards when the tethered hornets or

butterflies were held 10, 20, 30, 40, 50, 60 and 70 cm away from the entrance. For each test we followed the same protocol. The tethered test insect (hornet or butterfly) was presented to the colony 70 cm from the entrance. Here the test insect was held stationary for 10 s to alert the guards. We then moved the test insect forward towards the entrance (at a speed of about 100 cm/s), stopping for 10 s at each of the appropriate distances from the entrance in linear order.

In an independent test we held the test insect 10 cm from a colony entrance and moved it from side to side at two different speeds (estimated at 10 cm/s and 20 cm/s) through an arc of 50 cm for 20 s. Each trial was repeated on each of 3 days. The butterfly was always presented after the wasp. For each video recording of a particular colony, we recorded the frequency of the shaking in shakes/min for 10 randomly selected guards from each colony.

Analyses

Where possible we used ANOVA to analyse our data. When the assumption of homogeneity of variance was violated (Levene test of homogeneity of variance), we log transformed the data for significance testing, but present untransformed means and standard errors of the means. When the transformation did not stabilize the variance we used Mann–Whitney *U* tests to compare the variable of interest across bee species.

RESULTS

Free-flying Hornets

Apis mellifera colonies had a mean \pm SE of 79.7 ± 1.2 guard bees, about twice the number of guards that were seen in *A. cerana* colonies (42.6 ± 1.2 ; $F_{1,18} = 524.3$, $P < 0.001$). Despite the greater number of guards at the entrances of *A. mellifera* hives, untethered hornets ($N = 100$) remained a mean of 16.6 ± 0.4 cm away from the entrance of *A. cerana* hives and 10.4 ± 0.4 cm ($N = 100$) from *A. mellifera* hives ($F_{1,3,9} = 11.7$, $P = 0.027$, log transformed). However the mean distance at which hornets first approached *A. mellifera* colonies (12.8 ± 0.19 cm) was similar ($F_{1,1,65} = 0.25$, $P = 0.68$) to the first approach to *A. cerana* colonies (13.02 ± 0.19 cm), suggesting that they were repelled by the shimmering of the *A. cerana* guards. Hornet hovering time was significantly longer (two-tailed Mann–Whitney *U* test: $U = 8723.5$, $P = 0.001$, $N = 100$ hornet observations per honeybee species) at the entrance of *A. cerana* hives (59.9 ± 3.6 s) than at the entrance of *A. mellifera* hives (40.8 ± 3.6 s). Hornets were more likely to capture a bee at the entrance of *A. mellifera* hives (40% of hornet hoverings) than at the entrance of *A. cerana* hives (17%). Under a null hypothesis that a hornet would be equally likely to catch a bee of each species, this is significantly different (2×2 contingency table: $\chi^2_1 = 18.3$, $P < 0.001$).

Tethered Test Insects

The frequency of abdominal shaking (swings/min) by *A. cerana* guards (averaged across all days and colonies) increased strongly with decreasing distance at which tethered hornets were held in front of colonies (Fig. 1). In contrast, guards responded to the presence of a butterfly only when they were very close to the nest entrance, and the frequency of shaking was lower than the response to a hornet (Fig. 1). We analysed these data as a repeated measures ANOVA with colony and threat species (hornet or butterfly) as factors. Averaged over all distances the guards shook 32.1 ± 0.55 times/min in the presence of the tethered hornets and 1.13 ± 0.55 times in the presence of tethered butterflies. The effect of colony was not significant ($F_{9,180} = 0.48$, $P = 0.88$), and there was

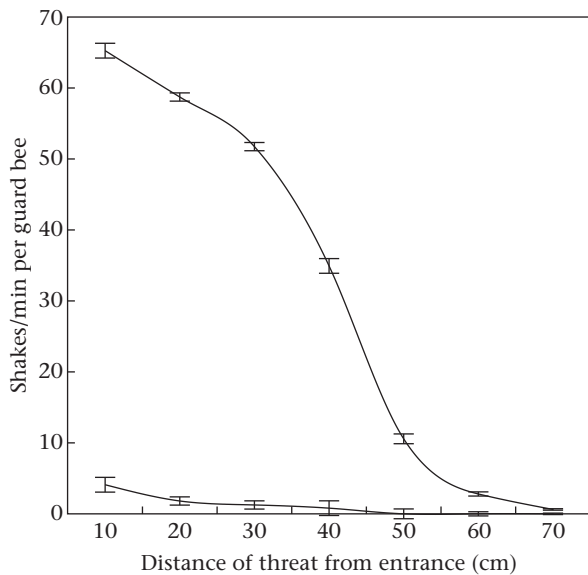


Figure 1. The frequency (shakes/min) of abdominal shaking by *A. cerana* guard bees with respect to the distance of tethered *Vespa velutina* (upper line) and *Papilio xuthus* (lower line). The error bars are the standard error of the means.

a highly significant interaction between threat species and distance ($F_{3,97,713.97} = 869.56$, $P < 0.001$, with Huynh–Feldt degrees adjustment for sphericity).

When *A. cerana* guards were confronted by a hornet moving at 10 cm/s 10 cm from their nest entrance, the mean frequency of abdominal shaking was 38.81 ± 0.66 shakes/min. When confronted by a butterfly moving at the same speed the mean frequency of abdominal shaking was 2.64 ± 0.66 shakes/min. The effect of species was highly significant (two-tailed Mann–Whitney U test: $U = 14922.5$, $P < 0.001$, $N = 100$ threat presentations per threat species). When we moved the threat at a speed of 20 cm/s 10 cm in front of the colony, the mean number of shaking movements was 2.53 ± 0.66 shakes/min for butterflies and 57.85 ± 0.66 for hornets. The effect of species was highly significant (Mann–Whitney U test: $U = 15\,048.0$, $P < 0.001$, $N = 100$ threat presentations per threat species).

DISCUSSION

Shaking by *A. cerana* guards appears to be an ISY display that significantly reduces the approach of the hornet *V. velutina* and reduces bee predation. The behaviour of bees and hornet fulfils all four of our key predictions if the shaking is indeed a coevolved ISY display between bee and hornet.

(1) The intensity of the shaking display should increase with proximity of the hornet to the nest. The frequency of abdominal shakes by *A. cerana* guards was correlated with a hornet's proximity and speed of movement, meaning that the intensity of the ISY is matched to the intensity of the threat. This modulated change in shaking frequency fulfils a prediction of the 'risk sensitivity hypothesis' (Helfman 1989), which argues that prey species should adjust their defensive behaviour according to the intensity of the predator's threat.

(2) Hornets should be repelled by the display. As a hornet approaches the nest entrance, the bees shake, and the hornet retreats and tries to catch flying bees as they return to the colony (Kloft & Schneider 1969; Abrol 2006; Schmelzer & Kastberger 2009; Fuchs & Tautz 2011; see Supplementary video). Thus it is clear that hornets are repelled by the signal, even though they remain in the

vicinity of an *A. cerana* colony longer than they do in front of an *A. mellifera* colony. *Vespa velutina* never lands on an *A. cerana* entrance when shaking is in progress and only catches flying bees (K. Tan, personal observations).

(3) The display should decrease bee predation. Lack of shaking by *A. mellifera* guards probably explains the higher frequency of bee kills in *A. mellifera* colonies and the shorter hovering time of hornets in front of *A. mellifera* colonies relative to *A. cerana* colonies, and the closer distance at which hornets hunt in front of *A. mellifera* nests relative to *A. cerana* nests. The hornets are more likely to make a kill at *A. mellifera* colonies and move on.

(4) *Apis cerana* guards should not respond with the shaking signal when approached by a nonthreatening species. The guards barely responded to the presence of a butterfly moving in front of their colony as might a hornet. A tethered hornet, moving at a similar speed to a tethered butterfly, elicited a strong shaking response in the guards. This striking difference strongly suggests that the guards recognize hornets, and distinguish them from nonthreatening organisms in their environment. The *A. cerana* guards reserve their display only for true threats. *Vespa velutina* responds to the display by not approaching the colony.

We do not wish to suggest that *A. cerana* uses its shaking display solely to repel *V. velutina*. The shaking display is used to deter nonhornet intruders, and is used in response to the presence of other species of hornet (e.g. Abrol 2006). However, we suggest that the intensity of the display, which is tuned to the movement and distance of a hornet, and the reduced response to a butterfly species that was both larger than a hornet and conspicuously coloured is evidence that the primary function of the display is to deter hornets.

Caro (1995) argued that there is a lack of convincing empirical evidence for pursuit-deterrent signalling in animals. For example, in some studies the 'predator' is the human investigator or a stuffed animal. Such studies can only show the behaviour of the 'prey', and not the response of the predator to the prey's signal. In contrast, the shaking behaviour of *A. cerana* and *A. nuluensis* (Koeniger et al. 1996) and the correlated response of the hornet predator (Schneider & Kloft 1971; Butler 1974; Koeniger et al. 2010; this study) strongly suggest that hornets move back from the colony entrance in response to the guard's display, thus providing an example of a predator responding to a signal from the prey. It is likely that the 'Mexican wave' displays of the open-nesting honeybee species *Apis andreniformis*, *Apis florea*, *Apis dorsata* and *Apis laboriosa* (Kastberger & Sharma 2000; Oldroyd & Wongsiri 2006; Schmelzer & Kastberger 2009) towards hornets and other predators are also ISY displays, although we only have preliminary evidence that these displays are backed up by defensive action such as heat balling (Kastberger & Stachl 2003). Importantly, Kastberger et al. (2008) showed by frame-by-frame analysis of interactions between the giant honeybee, *A. dorsata*, and predatory hornets, that hornets respond to the Mexican wave display by moving away from the nest. Thus the hypothesis that a hornet predator can be deterred by a prey's signal is well established in honeybees.

We conclude that the signals between Asian honeybees and their hornet predators are good examples of ISY displays, which could be useful model systems for further studying predator–prey signalling. For example, our experiments do not reveal what features the bees use to recognize hornets as a threat. The cues could be visual, olfactory or behavioural. Studies with models could help to elucidate the cues used.

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Supplementary material

Supplementary material associated with this article is available, in the online version, at doi:10.1016/j.anbehav.2011.12.031.

References

- Abrol, D. P.** 2006. Defensive behaviour of *Apis cerana* F. against predatory wasps. *Journal of Apicultural Science*, **50**, 39–46.
- Bergstrom, C. T. & Lachmann, M.** 2001. Alarm calls as costly signals of anti-predatory vigilance: the watchful babbler game. *Animal Behaviour*, **61**, 535–543.
- Butler, C. G.** 1974. *The World of the Honeybee*. London: Collins.
- Caro, T. M.** 1986. The functions of stotting in Thomson's gazelles: some tests of the predictions. *Animal Behaviour*, **34**, 663–684.
- Caro, T. M.** 1995. Pursuit-deterrence revisited. *Trends in Ecology & Evolution*, **10**, 500–503.
- FitzGibbon, C. D. & Fanshawe, J. H.** 1988. Stotting in Thomson's gazelles: an honest signal of condition. *Behavioral Ecology and Sociobiology*, **23**, 69–74.
- Fuchs, S. & Tautz, J.** 2011. Colony defence and natural enemies. In: *Honeybees of Asia* (Ed. by H. R. Hepburn & S. E. Radloff), pp. 369–395. Heidelberg: Springer.
- Hasson, O.** 1991. Pursuit-deterrent signals: communication between prey and predator. *Trends in Ecology & Evolution*, **6**, 325–329.
- Helfman, G. S.** 1989. Threat-sensitive predator avoidance in damselfish-trumpet fish interactions. *Behavioral Ecology and Sociobiology*, **24**, 47–58.
- Hirth, D. H. & McCullough, D. R.** 1977. Evolution of alarm signals in ungulates with special reference to white-tailed deer. *American Naturalist*, **111**, 31–42.
- Kastberger, G. & Sharma, D. K.** 2000. The predator–prey interaction between blue-bearded bee eaters (*Nyctornis aethoni* Jardine and Selby 1830) and giant honey bees (*Apis dorsata* Fabricius 1798). *Apidologie*, **31**, 727–736.
- Kastberger, G. & Stachl, R.** 2003. Infrared imaging technology and biological applications. *Behavior Research Methods, Instruments and Computers*, **35**, 429–439.
- Kastberger, G., Schmelzer, E. & Kranner, I.** 2008. Social waves in giant honeybees repel hornets. *PLoS ONE*, **3**, e3141. doi:10.1371/journal.pone.0003141.
- Ken, T., Hepburn, H. R., Radloff, S. E., 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.
- Koeniger, N., Koeniger, G., Gries, M., Tingek, S. & Kelitu, A.** 1996. Observations on colony defense of *Apis nuluensis* Tingek, Koeniger and Koeniger, 1996 and predatory behavior of the hornet, *Vespa multimaclulata* Perez, 1910. *Apidologie*, **27**, 341–352.
- Koeniger, N., Koeniger, G. & Tingek, S.** 2010. *Honey Bees of Borneo*. Kota Kinabalu: Natural History Publications (Borneo).
- Oldroyd, B. P. & Wongsiri, S.** 2006. *Asian Honey Bees. Biology, Conservation and Human Interactions*. Cambridge, Massachusetts: Harvard University Press.
- 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.
- Ruxton, G. D., Sherratt, T. N. & Speed, M. P.** 2004. *Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals and Mimicry*. Oxford: Oxford University Press.
- Sakagami, S. F.** 1960. Preliminary report on the specific differences in behaviour and other ecological characters between European and Japanese honey bees. *Acta Hymenopterologica*, **1**, 171–198.
- Schmelzer, E. & Kastberger, G.** 2009. 'Special agents' trigger social waves in giant honeybees (*Apis dorsata*). *Naturwissenschaften*, **96**, 1431–1441.
- Schneider, P. & Kloft, W.** 1971. Beobachtungen zum Gruppenverteidigungsverhalten der östlichen Honigbiene. *Apis Cerana. Zeitschrift Für Tierpsychologie*, **29**, 337–342.
- Smythe, N.** 1970. On the existence of 'pursuit invitation' signals in mammals. *American Naturalist*, **104**, 491–494.
- Smythe, N.** 1977. The function of mammalian alarm advertising: social signals of pursuit invitation? *American Naturalist*, **111**, 191–194.
- Tan, K., Li, H., Yang, M. X., Hepburn, H. R. & Radloff, S. E.** 2010. Wasp hawking induces endothermic heat production in guard bees. *Journal of Insect Science*, **10**, 142. insectscience.org/10.142.
- Vega-Renondo, F. & Hasson, O.** 1993. A game-theoretic model of predator–prey signalling. *Journal of Theoretical Biology*, **162**, 309–319.
- Walther, F. R.** 1969. Flight behaviour and avoidance of predators in Thomson's gazelle (*Gazella thomsoni* Guenther 1884). *Behaviour*, **34**, 184–221.
- Woodland, D. J., Jaafar, Z. & Knight, M.-L.** 1980. The 'pursuit deterrent' function of alarm signals. *American Naturalist*, **115**, 748–753.