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Giant Asian honeybees use olfactory eavesdropping to detect and avoid ant predators

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Pollinators provide a key ecosystem service that can be influenced by predation and predator avoidance. However, it was unclear whether pollinators can avoid predators by eavesdropping, intercepting predator signals. Using a natural species assemblage, we show that a bee can eavesdrop on and avoid the trail pheromone of a sympatric ant, while foraging on a native plant. The giant Asian honeybee, *Apis dorsata*, avoided *Calliandra haematocephala* inflorescences with live weaver ants, *Oecophylla smaragdina*. Although few foraging bees were attacked, ants killed the bee in almost a third of attacks. Ant presence alone significantly reduced bee floral visits. Bees showed nearly equal avoidance of live ants and trail pheromone extracts, demonstrating that olfactory eavesdropping alone can elicit full avoidance. We then used GC-MS to analyse compounds deposited by ants walking and laying trail pheromone. The most abundant compounds were all trail pheromone components. However, bees did not avoid the most abundant and conspicuous trail pheromone compound, heneicosane. Foragers may instead detect a mixture of different trail pheromone compounds. Our results contribute to a growing understanding of how public information about predators and competitors can shape food webs, and show that pollinators can tap into the private signals of predators and use this information to their advantage.

Predators can influence pollinator behaviour (Romero, Antiqueira, & Koricheva, 2011) and thereby influence pollination (Dukas, 2005), a key ecosystem service (Klein et al., 2007; Vanbergen & Initiative, 2013). To avoid predators, pollinators can use public information, arising from foragers, predators and their interactions (Chittka & Leadbeater, 2005; Goodale & Nieh, 2012; Romero et al., 2011). This information usage has cascading consequences for plant–pollinator mutualisms because predators can deter pollinator visits, thereby reducing seed (Suttle, 2003) and fruit production (Dukas, 2005). Eavesdropping, a type of public information use, is defined as receivers intercepting and using signals designed for other senders (Peake, 2005). Eavesdropping is particularly interesting because it has consequences for signal evolution. Signals should evolve to balance the twin pressures of carrying information for intended receivers and escaping detection by unintended receivers. Thus, eavesdropping on predator signals by pollinators has implications for pollination ecology and signal evolution.

Ants interact with pollinators in complex ways (Gonzálvez, Santamaría, Corlett, & Rodríguez-Gironés, 2013; Wielgoss et al., 2013). They can compete for floral resources with pollinators, deterring them through interference competition, exploitation competition and predation (Rodríguez-Gironés, Gonzálvez, Llandres, Corlett, & Santamaría, 2013). Through exploitation competition, live *Lasius niger* ants reduced the average per flower foraging time of bumblebees, *Bombus terrestris*, on ant-infested flowers (Ballantyne & Willmer, 2012). Argentine ants, *Linepithema humile*, exhibited interference competition and attacked pollinators at morning glory plants and reduced seed set (Hanna et al., 2014). *Solenopsis xyloni* ants also used interference competition to deter bee pollinator visits, resulting in fruits with significantly fewer and smaller seeds (Ness, 2006). In many cases, the precise form of competition (interference competition, exploitation competition or both) is unclear. Argentine ant presence repelled cactus bees (*Diadasia* spp.) from visiting barrel cacti, decreasing the number of seeds per fruit (LeVan, Hung, McCann, Ludka, & Holway, 2014). *Pheidole megacephala* ants repelled native *Hylaeus* bees from flowers (Lach, 2008). Predation or the threat of predation can also affect pollinators. Weaver ants repelled *Nomia* bees from flowers (Gonzálvez et al., 2013), evidently by presenting a predation threat. Finally, ants, particularly the weaver ant, *Oecophylla smaragdina*

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(Rodríguez-Gironés et al., 2013), can directly prey upon pollinators such as Asian honeybees, *Apis dorsata*. Such predation should enhance the benefits of predator detection.

Pollinators can therefore identify visual and olfactory cues associated with predation (Abbott, 2006; Gonçalves-Souza, Omena, Souza, & Romero, 2008; Goodale & Nieh, 2012). For example, honeybees, *Apis mellifera*, can sense and avoid live crab spiders (Dukas & Morse, 2003), a freshly frozen crab spider (Dukas, 2001), a dried spider (Brechbühl, Kropf, & Bacher, 2010) or a live praying mantis (Bray & Nieh, 2014). Olfaction plays a role in such predator detection. Bees avoided flowers upon which a spider had walked and may have deposited spider odour (Reader, Higginson, Barnard, & Gilbert, 2006). Bray and Nieh (2014) showed that honeybee foragers will avoid an extract of mantis odour. These responses can be learned or innate, although evidence suggests that learning is more likely. Bumblebees were not inherently repelled by the odour trail marks of ants (*L. niger* and *Formica selysi*) but can learn to associate these odours with unprofitable food (Ballantyne & Willmer, 2012). In all of these cases, the odours avoided were cues, not signals that have evolved to convey information to intended receivers and, potentially, to thwart unintended receivers.

In fact, it remains unclear whether pollinators can eavesdrop on the odour trail pheromone signals produced by ants. Ant odour trails are also used by many ant species that prey upon pollinators (Hölldobler & Wilson, 1990). Pollinators should be able to eavesdrop on ant trail pheromones because these odour trails are extensive and therefore fairly conspicuous. Although not a pollinator, the herbivorous beetle *Rhyparida wallacei* detects and avoids *O. smaragdina* pheromone (Offenberg, Nielsen, MacIntosh, Havanon, & Aksornkoae, 2004). Cembrowski, Tan, Thomson, and Frederickson (2014) showed that bumblebees avoided artificial feeders with live ants. Bees also avoided feeders upon which ants had walked, depositing ant scent. This ant scent could consist of odour cues such as cuticular hydrocarbon (CH) cues deposited by ant tarsi, chemical signals such as trail pheromones, or both (Cembrowski et al., 2014).

Because bees have excellent olfaction, they can detect CH ‘footprint’ odour cues left by other foragers and learn to associate these traces with nectar-depleted flowers (Goulson, Stout, Langley, & Hughes, 2000; Leadbeater & Chittka, 2007; Witjes & Eltz, 2009; Yokoi & Fujisaki, 2008). However, we suspected that the signal components of trail pheromone would be far more abundant than CH cues. Trail pheromone should therefore be easier for eavesdroppers to detect because odour concentration matters. Honeybees most easily detect and learn the most abundant odour components in an odour mixture (Reinhard, Sinclair, Srinivasan, & Claudianos, 2010).

We therefore hypothesized that *A. dorsata* foragers would eavesdrop on and avoid recruitment odour trails of *O. smaragdina*. These species are sympatric. *Apis dorsata* ranges from western India throughout continental and oceanic Asia, including Sulawesi, Indonesia and the Philippines (Hepburn & Radloff, 2011; Oldroyd & Wongsiri, 2006). *Oecophylla smaragdina* is similarly found throughout most of the Asian tropics, from India to the Solomon Islands and Queensland, Australia (Hölldobler, 1983). This ant produces a conspicuous, long-lasting recruitment odour trail that can persist for approximately 3 days, remaining strong for at least 24 h (Jander & Jander, 1979). It actively hunts for pollinators on flowers, including honeybees, *Apis cerana* and *A. mellifera* (Rodríguez-Gironés et al., 2013). Chen and Li (2012) reported that *O. smaragdina* would prey upon foraging *A. dorsata*, and attacked bees produced alarm pheromone that deterred other bees from visiting the same flowers. However, this study did not test whether bees could avoid live ants alone or ant odours. Finally, Asian honeybees have evolved defences against this ant species. *Apis florea* workers create a sticky barrier that effectively isolates their nests from *O. smaragdina*, reinforcing this barrier upon detecting a weaver ant, but not after detecting another arboreal ant species (Duangphakdee, Koeniger, Koeniger, Wongsiri, & Deowanish, 2005). Asian honeybees may therefore have evolved another defence, olfactory eavesdropping.

Our goals were therefore to (1) determine whether ant presence (ant visual and olfactory stimuli) on an inflorescence could repel *A. dorsata* foragers, (2) test whether *A. dorsata* can use olfactory eavesdropping to avoid this ant's trail pheromone, and (3) chemically analyse *O. smaragdina* trail pheromone and test bee eavesdropping on the trail pheromone's most abundant chemical component.

METHODS

Field Observations

This research was conducted in full compliance with the laws of the People's Republic of China. No specific permits were required for our field studies, which were conducted at Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Sciences. Our study species: *A. dorsata* (bees), *O. smaragdina* (ants) and *Calliandra haematocephala* (plant) are not endangered.

The field experiments were conducted from February 2013 to April 2014, during the blooming season of *C. haematocephala*, a species that we chose because *O. smaragdina* preys upon *A. dorsata* foraging on *C. haematocephala* inflorescences (Chen & Li, 2012). These shrubs were abundant at our field site and often contained weaver ants, which we observed attacking and killing *A. dorsata* foragers. Each inflorescence of *C. haematocephala* is globose and consists of an average of 40 flowers whose numerous long slender stamens (approximately 25 per flower) create the ‘powder puff’ appearance (Fig. 1a) that gives this plant one of its common names (Nevling & Elias, 1971). These inflorescences attracted *A. dorsata* and *O. smaragdina*. At our site, weaver ants were fairly common (we found 52 colonies at XTBG), and we observed them foraging for nectar, attacking and capturing *A. dorsata* (Fig. 1). On these inflorescences, we observed ants exhibiting typical trail pheromone deposition behaviour: dragging their abdomens and depositing small visible trail pheromone spots (Offenberg, 2007).

We created two patches (each 3 × 10 m), one with ants and one that was ant-free. Each patch contained 10 small trees that were approximately 3 m tall. None of these trees contained any ants, based upon thorough visual inspections. The patches were separated by 5 m, and trees from one patch did not have branches that touched trees from the other patch. In the ant-treated patch, we physically connected the branches of each pair of trees and released five queenright colonies of *O. smaragdina*, collected from mango and pomelo trees in the nearby botanical garden, one colony per tree pair. We waited 1 week after introducing the ants for their colonies to become established and then began the experiments. No ants were added to the ant-free patch, and we applied rings of sticky Tanglefoot resin around the trunks and branches to keep ants off of these trees. During our experiments, we continued to meticulously inspect the ant-free trees and confirmed that they were ant free.

The *A. dorsata* foragers probably came from approximately 40 colonies located about 1 km away from our study site. We could not determine precisely how many different colonies came to our inflorescences because we used naturally foraging bees. However, we conducted our study over 15 months and used 20 different trees. We therefore probably used bees from multiple colonies.