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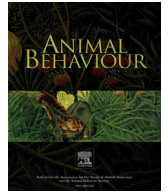
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Honeybees tune excitatory and inhibitory recruitment signalling to resource value and predation risk



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Group-foraging animals can coordinate their activities by performing positive signals that increase foraging or inhibitory signals that decrease recruitment when foragers detect danger. However, it is unclear whether foragers tune their excitatory and inhibitory signalling according to food value and predation risk. We therefore studied the signals that honeybee foragers perform before and after being attacked by live predators (wasps and spiders) or a robo-predator at a nectar source. Predator attacks significantly reduced recruitment dancing and increased stop signalling, which inhibits dancing for the dangerous resource. Attack equally reduced dancing for all sucrose concentrations. However, foragers factored travel costs into their positive signalling. At the feeder with greater travel cost (100 m), bees danced less when they responded more severely to attacks. At the low travel cost feeder (1 m), there was no significant effect of attack response severity upon dancing. Attacks increased inhibitory signal production. Live and robo-predator attacks elicited 131-fold more stop signals from foragers as compared to control treatments of freshly dead predators that did not attack. However, food profitability, distance and sucrose concentration did not alter stop signalling. We suggest that this pattern may generally characterize excitatory/inhibitory signal pairs in group foraging. Foragers tune positive signalling (recruitment) to food quality and peril, and this is countered by an inhibitory signal that is tuned to danger but not resource value.

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Signals have been shaped through natural selection to convey information and, in many cases, can excite by increasing actions or inhibit by decreasing actions. Signals that galvanize collective action for foraging are widespread and can rapidly mobilize the group to exploit profitable food sources (Jeanson & Deneubourg, 2009). For example, food-associated calls can increase foraging in chickens (Evans & Evans, 1999) and marmosets (Kitzmann & Caine, 2009). In contrast, warning signals provide inhibition. Such signals are often triggered by predators (Blumstein, 1999; Cäsar, Byrne, Young, & Zuberbühler, 2012; Clay, Smith, & Blumstein, 2012; Lemasson, Ouattara, Bouchet, & Zuberbühler, 2010), and can inhibit foraging in a wide variety of birds and mammals (Caro, 2005).

In some cases, the excitatory and inhibitory signals are tightly coupled in a pair, and this close linkage improves collective decision making. The pharaoh ant uses a 'no-entry' odour signal to indicate that a food odour trail is no longer rewarding (Robinson, Jackson, Holcombe, & Ratnieks, 2005). A treehopper mother can reduce

false alarms by producing a vibrational signal that inhibits the alarm signal vibrations produced by her offspring (Hamel & Coccoft, 2012). Honeybees possess a powerful excitatory signal, recruitment dancing, which can rapidly increase the number of workers visiting a resource (von Frisch, 1967). This recruitment signal is paired with a stop signal that inhibits dancing (Kirchner, 1993; Nieh, 1993; Pastor & Seeley, 2005; Seeley et al., 2012) when foragers experience conspecific attack (Nieh, 2010) or food source overcrowding (Lau & Nieh, 2009; Nieh, 1993; Thom, 2003). The stop signal is a 300–400 Hz vibrational signal with a duration of approximately 150 ms (Lau & Nieh, 2009; Seeley et al., 2012) that a worker usually delivers while butting its head into the body of the receiver, causing the receiver to momentarily freeze (Kietzman, 2015; Michelsen, Kirchner, & Lindauer, 1986; Nieh, 1993; Thom, Gilley, & Tautz, 2003). Although they can be triggered by peril, stop signals are not necessarily warning signals because they inhibit recruitment dancing in another context, house hunting (Seeley et al., 2012). In the contexts of foraging and house hunting, stop signals share a common function. They increase the speed of colony decision making by inhibiting recruitment.

Here, we examined the most common stop signal context, foraging, and determined the effect of predation and food quality on

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stop signals. To date, no studies have demonstrated that predator encounters elicit stop signals, although predation may be more common (Dukas, 2004; Morse, 1986) than conspecific fights over food or overcrowding on a food source. Nieh (2010) elicited stop signalling when bees from different colonies fought for food, smelled sting alarm pheromone, or were pinched with forceps. These latter two stimuli may also be experienced during predator attacks, but attacks by real predators are necessary to demonstrate that predators can elicit stop signals. In preliminary observations, we saw yellow-jacket wasps (*Vespa pensylvanica*) and green lynx spiders, *Peucetia viridans*, attacking foragers with varying degrees of severity, as gauged by predator behaviours and bee responses. We hypothesized that foragers would produce stop signals and increase stop signal production in response to more severe attacks from these predators.

The excitatory side of this signal pair, recruitment dancing, is strongly influenced by food value, and we therefore wondered whether stop signalling would also be affected by food quality. Bees perform more dance circuits and thereby increase recruitment (Seeley, Camazine, & Sneyd, 1991) for higher-quality (sweeter) nectar (Seeley, Mikheyev, & Pagano, 2000; Waddington, 1982) and for food that incurs a smaller travel cost by being closer to the nest (Seeley et al., 1991). Thus, food profitability influences the number of recruitment dance circuits (the number of excitatory signals) produced by a forager (Seeley et al., 1991). We therefore hypothesized that foragers would decrease the level of inhibitory signalling and produce fewer stop signals after attacks at more profitable as compared to less profitable food.

Finally, recruitment dancing is influenced by predation. Honeybees reduce recruitment dancing for dangerous foraging sites (Abbott & Dukas, 2009; Bray & Nieh, 2014). Attacks by conspecifics or by the simulated bites of a conspecific also reduce honeybee recruitment dancing (Nieh, 2010). However, it not clear whether dancers adjust the level of recruitment dancing according to the severity of predator attack and whether they weigh food profitability against attacks. If the food is sufficiently profitable, will nonlethal attacks affect recruitment dancing?

We therefore tested the effects of attacks from live predators (wasps and spiders) and a robo-predator designed to provide a very consistent attack stimulus on honeybee recruitment dancing and stop signalling, an excitatory/inhibitory pair of signals. We sought to determine (1) whether live predator attacks can elicit inhibitory signalling in bees, (2) whether attack severity correlates with increased stop signalling and decreased recruitment dancing and (3) whether foragers consider food profit in modulating their level of excitatory or inhibitory signalling after predator attacks, discounting attacks if the food is very profitable.

METHODS

Study Site and Colonies

We conducted our study at the University of California San Diego Biological Field Station (BFS) in La Jolla, California from May to November of 2011. We used two three-frame observation colonies of *Apis mellifera ligustica*, each containing approximately 5000 bees. We censused colonies by counting photos of representative 65 cm² comb sections (modification of Imdorf, Buehlmann, Gerig, Kilchenmann, & Wille, 1987). Each colony had an egg-laying queen, a full comb of brood and an upper comb full of capped honey. Hives were housed in a temperature-controlled room (32 °C) to preserve normal hive temperatures while one side was open to record sounds. Hive monitors were ventilated bee suits. A 0.5 m long (2.5 cm inner diameter) vinyl entrance and exit tube connected the hive to the exterior. An internal metal slide directed bees to one side, where they danced and produced stop signals

(Nieh, 2010). A room window opened during observations provided illumination and allowed some bees to leave the hive. However, after becoming accustomed to the open hive, most bees entered and exited through the tube.

General Methods

We trained foragers to an inverted-jar feeder with a grooved bottom plate (von Frisch, 1967) that provided unscented sucrose solution on a 1 m high tripod. During training, we used 2.0 M sucrose solution (55% w/w) to elicit recruitment. Each feeder-visiting bee was marked on its thorax or abdomen with a unique combination of enamel paints. We verified that each bee at our feeder was from the focal observation hive by checking for its return to the hive. We removed all other bees with an aspirator. Bees were considered trained once they made at least 10 feeder visits.

To examine the effect of food profitability on stop signal production, we manipulated two variables: feeder sucrose concentration and distance to the feeder. After training, we replaced the 2.0 M sucrose solution with a solution of a randomly chosen test concentration: 0.5 M, 1.0 M, 1.5 M or 2.5 M, corresponding to 16%, 31%, 43% and 65% sucrose (w/w), respectively. We chose these concentrations to represent a wide natural range of nectar sugar concentrations. Generalist bee foragers, like honeybees, collect nectars ranging from 10 to 70% sugar (w/w) (Roubik, Yanega, Aluja, Buchmann, & Inouye, 1995). Before measuring bee behaviour, we allowed foragers to make five collecting trips to adjust to the new sucrose concentration. Bees were trained to feeders 1 m or 100 m away from the focal colony. Honeybees usually forage and recruit for natural food at much greater distances (Couvillon, Schürch, & Ratnieks, 2014; Waddington, Herbert, Visscher, & Richter, 1994), but we chose these distances because 100 m was the furthest distance to which we could reliably train bees for all the tested sucrose concentrations.

Bees perform recruitment dances that are called round dances when the food is close to the nest and waggle dances when the food is approximately >100 m away from the nest (von Frisch, 1967). However, both round and waggle dances are part of a continuum of recruitment dances and, in both cases, the number of bees recruited correlates with the number of dance circuits performed (Gardner, Seeley, & Calderone, 2008). We therefore used the general term 'recruitment dancing' to describe both dance types.

Experiments consisted of monitoring individual foragers before and after they received a treatment (Nieh, 2010). In the 'before' phase, we randomly selected a forager that had just returned to the hive. A hive visit began when a forager first walked onto the comb above the hive entrance and ended when it exited the hive through the hive entrance or by flying away. Once the bee returned to the feeder, we applied a treatment: attack by a (1) live spider, (2) live wasp or (3) robo-predator, or control exposure to a freshly dead (4) spider or (5) wasp. Each bee received only one treatment and was used for only one pair of observations. The 'after' phase began when this bee returned to the hive after the treatment.

We recorded bee behaviour and sounds with a video camera (Sony HD-HC7, New York, NY, U.S.A.). We manually tracked the focal bee with a microphone (Radio Shack model number 33-3013 Fort Worth, TX, U.S.A.) attached to a 30 cm rod held approximately 1 cm above its thorax (Nieh, 2010). The microphone was amplified with a microphone preamp (RTS Systems model number 132170 Burbank, CA, U.S.A.) whose output was routed through the video camera to headphones. The observer recorded (1) time until the bee first unloaded its food for >1 s with a digital timer (unloading time, Seeley, 1992), (2) the number of dance circuits and (3) the number of stop signals. A stop signal can be recognized by its distinctive sound, a brief vibrational pulse (170 ms) at approximately 360 Hz (Lau & Nieh, 2009), and by the signaller's behaviour, a signaller

typically lunging forward with its head to vibrate a bee that freezes during the signal (Michelsen, 2014; Nieh, 2010).

While the hive observer recorded this information, the feeder monitors used an aspirator to carefully capture all other bees visiting the feeder so that they would not be exposed to stimuli associated with the treatment. Any unmarked bees were subsequently marked, and all bees were released at the end of each trial day to avoid depleting the colony of foragers.

Preparing and Presenting Predators

At our field site, we observed green lynx spiders (*P. viridans*) attacking bees on flowers and western yellowjackets (*V. pensylvanica*) attacking honeybees on feeders. *Peucetia viridans* (Oxyopidae) occurs throughout the southern United States, Mexico and Central America, and often preys upon pollinators, including honeybees, by waiting on inflorescences (Louda, 1982; Weems & Whitcomb, 2001). *Vespa pensylvanica* occurs naturally west of the Rocky Mountains from southern California to southwestern Canada (Visscher & Vetter, 2003) and can attack and capture live honeybees (Wilson & Holway, 2010). During preliminary observations, we observed 20 *V. pensylvanica* wasps performing 116 attacks on honeybees at feeders. These attacks ranged in severity from touching to grabbing and biting (four trials, 2 h total observation time). We therefore used these species as a predator.

Plastic vials were used to capture spiders on flowers, and insect nets were used to capture wasps at the nest entrances of a separate set of honeybee colonies. We never observed spiders or wasps near the nest entrances of our observation colonies. To handle predators, we used cyanoacrylate glue to attach a 6 × 0.5 cm paper tether to each wasp thorax and spider abdomen (Fig. 1a, b). To immobilize the wasps for tethering, we chilled them on ice for approximately 2 min. Spiders did not survive chilling, so we placed a spider in a small cup, applied the glue to the paper tether and used forceps to quickly lower the tether onto its abdomen while it was moving. Compensating for the spider's motion was tricky but possible with practise. After tethering, wasps and spiders were allowed to recover in a container for 30 min before use. On a given day, we used a different individual predator for each attack, and then returned the predator to a holding container for the next experimental day. Each predator was used approximately twice. In the holding container, wasps were given 1.0 M sugar solution, but spiders were not fed. Control spiders and wasps consisted of dead specimens that we collected separately. We froze these live controls for approximately 10 min before control trials to preserve their odour and appearance and then tethered them. To avoid having odours like honeybee alarm pheromone on the predators, we did not expose these control predators to honeybees before using them and we used each control only once.

We used locking forceps to present the spiders and wasps. Once the focal bee landed, we approached it on its posterior right side with a predator and maintained an initial separation of approximately 3 mm. We allowed the living predator to initiate contact with the bee. An attack was defined as a predator contacting the bee. We chose an attack duration of 10 s, defined as the time that a predator contacted a bee, because this was the approximate duration of natural *V. pensylvanica* attacks on honeybees at a feeder at our field site (R. T. Jack-McCollough & J. C. Nieh, personal observation). If a bee moved to a new position on the feeder or flew away and subsequently returned, we again placed the predator 3 mm away from the bee and allowed it to continue its attack for a total of 10 s. Typically, we placed the predator on the feeder for 10–15 s to elicit a 10 s forager attack. Predators usually began an attack within a few seconds of being placed close to the focal bee.

Honeybees that stung subsequently died. We did not use data from bees that stung predators because these bees did not return to

the nest to perform recruitment dances or produce stop signals. We therefore tried to avoid fatal attacks and pulled the predator back if a deadly encounter was about to occur: a bee about to sting the predator, a wasp about to sting the bee, or a spider about to bite the bee. This method of preventing fatal attacks generally worked: only 3% of bees died.

One feeder observer provided the predator treatment and a second one recorded the number and type of predator attacks and the bee's responses at the feeder. During control trials, we placed a freshly dead predator at ambient air temperature also approximately 3 mm on the posterior right side of bee. Bees sometimes turned to face the predator and could then see and smell it. We never allowed the bee to contact the control predator and kept the control predator 3 mm away from the bee.

Scoring Live Predator Attack Severity

We measured the effect of predator attack severity on forager behaviour by counting the number of predator attacks. The predator initiated all attacks because it was presented 3 mm away from the bee. An attack consisted of the predator moving itself closer to the bee to perform one or more of the following behaviours: (1) 'touching': making brief contact, usually <2 s; (2) 'grabbing': hooking a leg onto the bee and drawing it in; (3) 'biting': making contact with mouthparts but not necessarily piercing the cuticle, which could be fatal with a spider predator. Touching was likely an attack behaviour, not simply the result of the predator attempting to escape, because touching was always followed by at least one other clear attack behaviour (grabbing or biting). These attack behaviours are similar to those observed by Rangel, Griffin, and Seeley (2010) between honeybee workers freely fighting over a nest site. Although predators were tethered in our trials, they showed similar attack behaviours to untethered free-flying wasps and spiders.

Each predator often made multiple attacks on the same bee. Some attacks could be interpreted as more severe (grabbing or biting as compared to touching), but there was considerable variation in how intensely predators made these attacks, and it was difficult for observers to judge attack force. For example, touching or biting could be forceful or light. To consider these attacks objectively, we defined attack severity as the sum of all predator attacks during an attack bout. For instance, an attack bout with two attacks was given an attack severity score of 2. This scoring equally weighted each type of attack, but provided a more conservative measure because the observer did not subjectively classify one type of attack as more intense than another.

On the feeder, we measured four bee responses to attack: (1) 'moving away': a bee walked to a different part of the feeder; (2) 'flying away': a bee flew away; (3) 'grappling': a bee fought back and used its legs to hold onto the predator; (4) 'attempting to sting': a bee arched its abdomen and pressed its stinger against the predator. Attacks sometimes elicited multiple bee responses. We defined bee response severity as the total number of bee responses on the feeder to a predator attack bout. For example, a bee that responded twice during a predator attack bout had a response severity score of 2. This scoring equally weighted each type of response, but had the advantage that the observer did not subjectively classify one type of response as more severe than another. Response severity only referred to the immediate responses of bees on the feeder, not to stop signal production inside the nest.

Electromechanical Predator

Because live predator attacks varied in severity, we built an electromechanical 'robo-predator' (Fig. 1c) to test the effects of attacks with a consistent bite force on bee recruitment dancing and

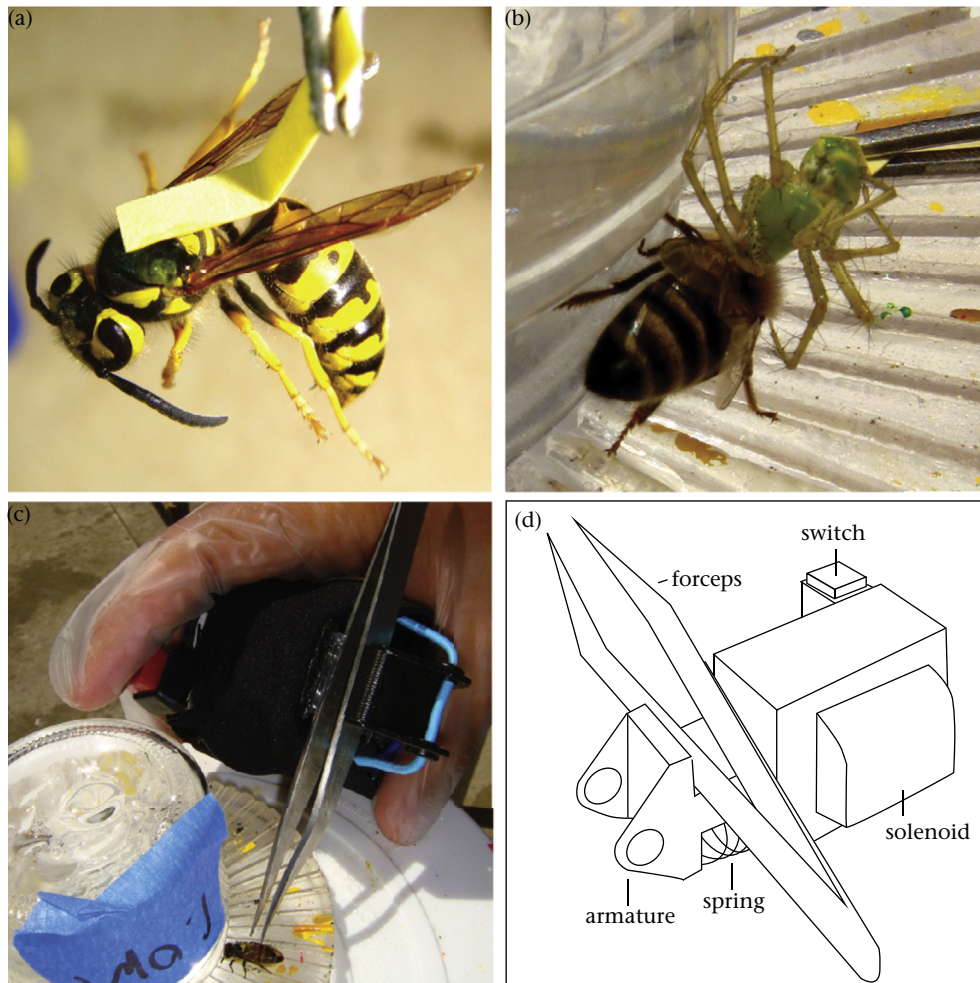


Figure 1. Details of using live predators and the electromechanical predator to attack bees. (a) Photo of the paper tether attached to the wasp (*V. pensylvanica*) thorax to control its attacks on bees. (b) A spider (*P. viridans*) attacking a bee. In this photo, the spider was presented on the right side of the bee (see [Methods](#)), but shifted its position during the attack. (c) The electromechanical predator posed to attack a bee. (d) Details of the electromechanical predator. The momentary switch allowed current to flow through the solenoid, drawing in the armature, which closed the forceps. The spring returned the forceps to the open position when there was no current. Pinching force was proportional to current, which was controlled by a rheostat (not shown) and calibrated (see [Methods](#)). The device was covered with protective neoprene insulation, and an elastic cord (shown as light blue in panel c) prevented the armature from extending too far.

stop signal production. Unlike living predators, the robo-predator always attacked with the same intensity and did not alter its attack in response to bee counterattacks. We therefore did not use the robo-predator to test how bees interact on the feeder with predators.

Our preliminary observations suggested that most wasps attacked by biting. To mimic predator biting, we custom-modified an electromechanical CKD AS-ORN solenoid coil (CKD Corp., Komaki, Aichi, Japan, [Fig. 1d](#)) that drew in an arm, thereby squeezing forceps to exert a bite force proportional to the current supplied (modulated with a rheostat). A momentary switch allowed the operator to activate the robo-predator (10 s attack/bee). We calibrated this device by using the forceps to pinch a 1 mm wide flat metal bar (matching the width of a bee's metathoracic basitarsus) attached across the centre of an Arduino piezoelectric ceramic disc sensor (20 mm diameter, www.arduino.cc). We generated a calibration curve by adjusting the rheostat to give varying levels of force and measured the sensor voltage with a digital oscilloscope (TDS2024B, Tektronix Inc., Beaverton, OR, U.S.A.). To determine the actual force levels, we modified the procedure of [Tautz, Roces, and Holldobler \(1995\)](#). We measured force with a type 8001 impedance head attached to a type 4294 vibrational calibrator (Brüel &

Kjaer, Norcross, GA, U.S.A.). The impedance head was pressed against the centre of the metal bar on the sensor disc, which in turn rested on a polyurethane foam base attached to a height-adjustable stand. As the stand was raised, the vibrational calibrator applied more force to the disc sensor via the impedance head, allowing us to calibrate the sensor disc voltages with actual force levels measured by the impedance head. We could then determine the force levels applied by the robo-predator forceps.

The average bite force of a predator attacking a honeybee has not been measured, but leaf-cutting ant mandibles exert forces of 10–20 mN_{pk-pk} for soft leaves and 50–100 mN_{pk-pk} for tough leaves ([Tautz et al., 1995](#)). We set our robo-predator to provide average forces of 150 ± 29 mN_{pk-pk} (calibrated at the beginning of each trial), the minimum force required to elicit sting extension in more than 97% of bees. No bees that were pinched with this level of force suffered evident harm. They continued to walk normally on the feeder and could waggle dance inside the nest, a task that requires complex motor coordination of leg movements ([Landgraf, Rojas, Nguyen, Kriegel, & Stettin, 2011](#)). To attack bees, we positioned the forceps around the right metathoracic leg at the basitarsus ([Fig. 1c](#)) because our preliminary observations showed that wasps often attacked by biting bee legs. After each treatment, we carefully

used laboratory detergent and 100% ethanol to clean the robo-predator, the feeder and the feeder platform to remove potential odours.

Statistics

All data were analysed using JMP v.9 software (SAS Institute). We used analysis of variance (ANOVA, REML algorithm) with colony as a random effect. Based upon residuals analysis, we log transformed dance circuit and stop signal data to meet parametric assumptions and tested the fixed effects of the number of predator attacks, distance, sucrose concentration and predator type (dead spider, dead wasp, live spider, live wasp or robo-predator). We performed linear regression to test the effect of the number of predator attacks on bee flying-away responses because this was the most common response to predator attacks.

To determine the effect of sucrose concentration on dancing, we used the log-transformed number of dance circuits during the before phase. We tested the effect of treatment on log Δ dance circuits (number of dance circuits during the after phase – number of dance circuits during the before phase). We used a post hoc contrast test to investigate differences between control and attack treatments.

To test the effects of treatment on stop signal production, we tested the effect of predator type on log-transformed Δ stop signals (number of stop signals during the after phase – number of stop signals during the before phase). We used Tukey's honestly significant difference (HSD) tests to determine treatment differences (Zar, 1984). We began with full models that included interactions and eliminated them if they were nonsignificant ($P < 0.05$). Averages are reported as means \pm SD.

RESULTS

We tested 259 bees from two colonies (112 bees from colony 1, 147 bees from colony 2). These bees produced a total of 1066 stop signals (4.1 ± 13.0 stop signals/bee) and 1260 dance circuits. Before they unloaded food, bees waited a fairly short and uniform time inside the nest to unload their food: 9.9 ± 9.5 s before treatment and 14.1 ± 20.1 s after treatment.

Recruitment Dancing without Predation Encounters Increased with Sucrose Concentration but not Distance

Prior to treatment, as shown by other studies (Seeley et al., 2000; Waddington, 1982), the number of dance circuits performed per bee visit to the nest increased with sucrose concentration ($F_{1,255} = 8.07$, $P = 0.005$; Fig. 2). There was no effect of distance ($F_{1,254} = 0.72$, $P = 0.40$), no significant interaction of sucrose concentration*distance and colony accounted for 21% of model variance.

Wasp and Spider Attacks Altered Bee Behaviours at the Food Source

Wasps and spiders attacked with approximately equal severity. There was no significant overall difference between wasp or spider attack severity (attack severity: $F_{1,98} = 0.66$, $P = 0.42$). Spiders were never allowed to bite bees because this would result in immediate bee death. However, attempted biting only accounted for 3.5% of live predator attack behaviours ($N = 314$ total attack behaviours), which were dominated by grabbing (20.4%) and touching (76.1%). There were no significant differences between the number of grabs or touches by spiders or wasps ($F_{1,98} \geq 2.15$, $P \geq 0.15$). The dead predator control treatments did not elicit any aversive responses (0 ± 0 responses for wasps and spiders).

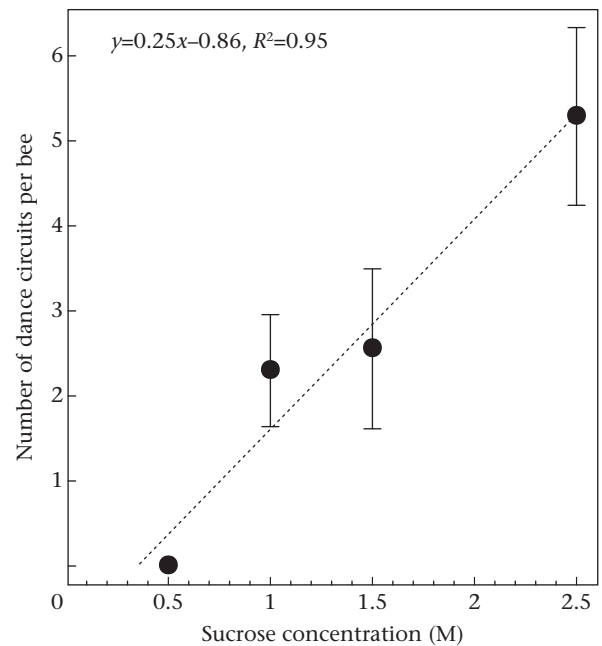


Figure 2. Effect of food quality (sucrose concentration) on recruitment dancing. Mean, standard error bars and linear regression line shown. Foragers collected, but did not dance for, 0.5 M sucrose solution.

Bees responded to live predator attacks by moving away (22.9% of responses), flying away (60.4%), grappling with the predator (15.7%) and attempting to sting the predator (1%; Fig. 3a). More severe predator attacks elicited more bee responses on the feeder ($F_{1,85} = 73.67$, $P < 0.001$; Fig. 3b). There were no significant effects of sucrose ($F_{1,94} = 0.08$, $P = 0.78$), distance ($F_{1,4} = 0.41$, $P = 0.55$) or predator type ($F_{1,63} = 0.94$, $P = 0.34$) upon bee response severity. All interactions were nonsignificant, and colony accounted for less than 1% of model variance.

We then focused on the most common response to predator attack, the bee flying away (Fig. 3a). More severe predator attacks resulted in an increased probability of bees flying away ($F_{1,71} = 22.86$, $P < 0.0001$), an effect largely due to wasp attacks. Wasps elicited a stronger bee response than spiders (predator type*attack severity interaction: $F_{1,94} = 6.44$, $P = 0.01$). The rate of bee departure was 2.7-fold higher (comparing regression slopes) for wasp attacks than for spider attacks (Fig. 3c, d). The increase in the bees' flying away in response to more severe attacks was strongly influenced by a single outlier for spider attacks (Fig. 3c, $R^2 = 0.19$), but was more robust for wasp attacks (Fig. 3d, $R^2 = 0.48$). There were no significant effects of sucrose ($F_{1,93} = 0.63$, $P = 0.43$), distance ($F_{1,1} = 0.29$, $P = 0.70$), or any interactions. Colony accounted for less than 1% of model variance. More severe predator attacks therefore led to more bee responses.

Predator Attacks Reduced Recruitment Dancing

Overall, predator attacks significantly reduced recruitment dancing (Δ dance circuits) by an average of four dance circuits per performance (predator type effect: $F_{1,250} = 2.56$, $P = 0.039$; Fig. 4a). There were no significant effects of sucrose ($F_{1,252} = 0.001$, $P = 0.98$) or distance ($F_{1,40} = 1.23$, $P = 0.27$) on Δ dance circuits. Recruitment dancing significantly decreased in bees that were attacked (spider, wasp or robo-spider) as compared to controls (dead spider, dead wasp; post hoc contrast test: $F_{1,252} = 6.28$, $P = 0.01$). All interactions were nonsignificant and colony accounted for less than 1% of model variance.

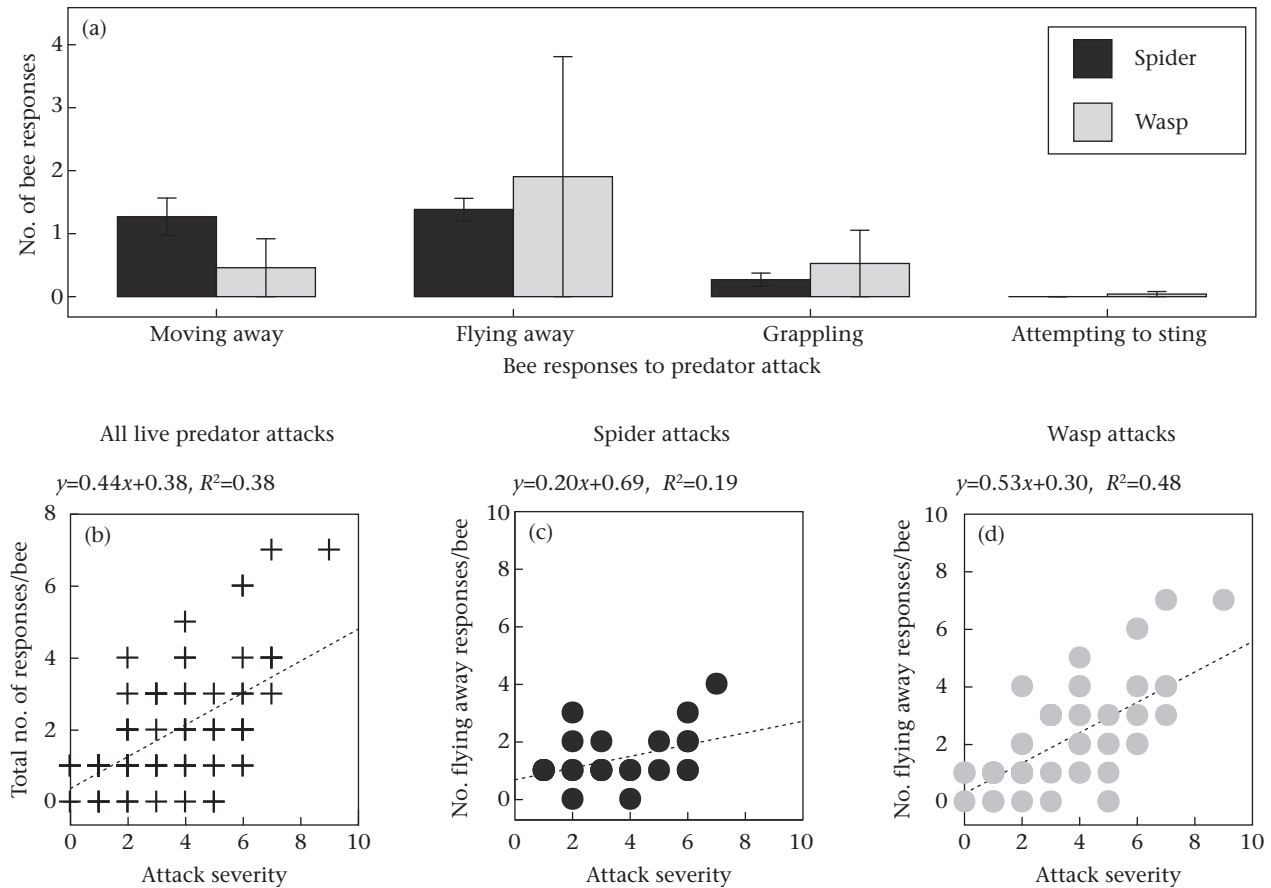


Figure 3. Responses of bees on the food source to live predator attacks. (a) Distribution of responses to attackers (standard error bars shown). (b) Total bee responses per attack at different attack severity levels by spiders and wasps. The effect of attack severity by (c) spiders and (d) wasps on the most common bee response, flying away. Linear regression lines and equations are shown.

However, the model that considered the severity of bee responses on the feeder to predator attacks revealed a distance effect. In this model, predator attacks affected recruitment dancing only when the food was farther from the nest (Fig. 4b). At 100 m, bees that responded more severely also significantly reduced recruitment dancing (bee response severity effect: $F_{1,64} = 15.69$, $P = 0.0002$; sucrose effect and interaction both nonsignificant, and colony accounted for less than 1% of model variance). At 1 m, the effects of bee response severity, sucrose and the interaction response severity*sucrose were all nonsignificant.

Predator Attacks Increased Stop Signal Production

Predator attacks increased stop signalling by 131-fold overall (predator type effect: $F_{4,251} = 18.86$, $P < 0.0001$; Fig. 4c). Foragers that were given the control treatment, a dead predator, produced virtually no stop signals, generating only 0.04 ± 0.26 stop signals on average. There was no significant effect of sucrose ($F_{1,251} = 1.90$, $P = 0.17$) or distance ($F_{1,214} = 2.31$, $P = 0.13$), and colony accounted for 7.5% of model variance. All attack treatments resulted in significantly more stop signals than either control treatment (Tukey's HSD: $P < 0.05$; Fig. 4c). Stop signal production in bees that were attacked was not affected by bee response severity on the feeder ($F_{1,95} = 0.91$, $P = 0.34$), sucrose concentration ($F_{1,95} = 0.44$, $P = 0.51$) or distance ($F_{1,85} = 0.55$, $P = 0.46$). All interactions were nonsignificant and colony accounted for 11% of model variance.

DISCUSSION

The honeybee recruitment dance can allocate a large number of nestmates to a good food resource (von Frisch, 1967). However, as dancers recruit inside the nest, a discrepancy can develop between their older information and the knowledge of more recent foragers. We show that the predation information possessed by recent foragers has two effects. First, predatory attacks reduced overall recruitment dancing by attacked bees (Fig. 4a). Resource value influenced how predation attempts altered these recruitment decisions. Bees decreased their recruitment dancing according to how severely they responded to attacks on the feeder when the travel cost was higher (100 m), but not when it was lower (1 m; Fig. 4b), perhaps because the closer resource was so accessible and therefore valuable. Second, bees attacked by live and robo-predators produced stop signals (Fig. 4c), thereby inhibiting dancing by other bees with outdated information about the resource (Nieh, 2010). Focal bees that were attacked by a live predator or the robo-predator produced significantly fewer stop signals than bees that were exposed to dead predators, which did not attack. These data provide the first demonstration that decreases in honeybee recruitment dancing due to predator attacks are tuned to travel cost (distance) and that natural predators can elicit stop signals.

Bees responded more strongly on the feeder to more severe predator attacks (Fig. 3). We allowed the predators to initiate attack, but limited the maximum severity of attacks by pulling predators away before attacks resulted in bee death. Thus, natural

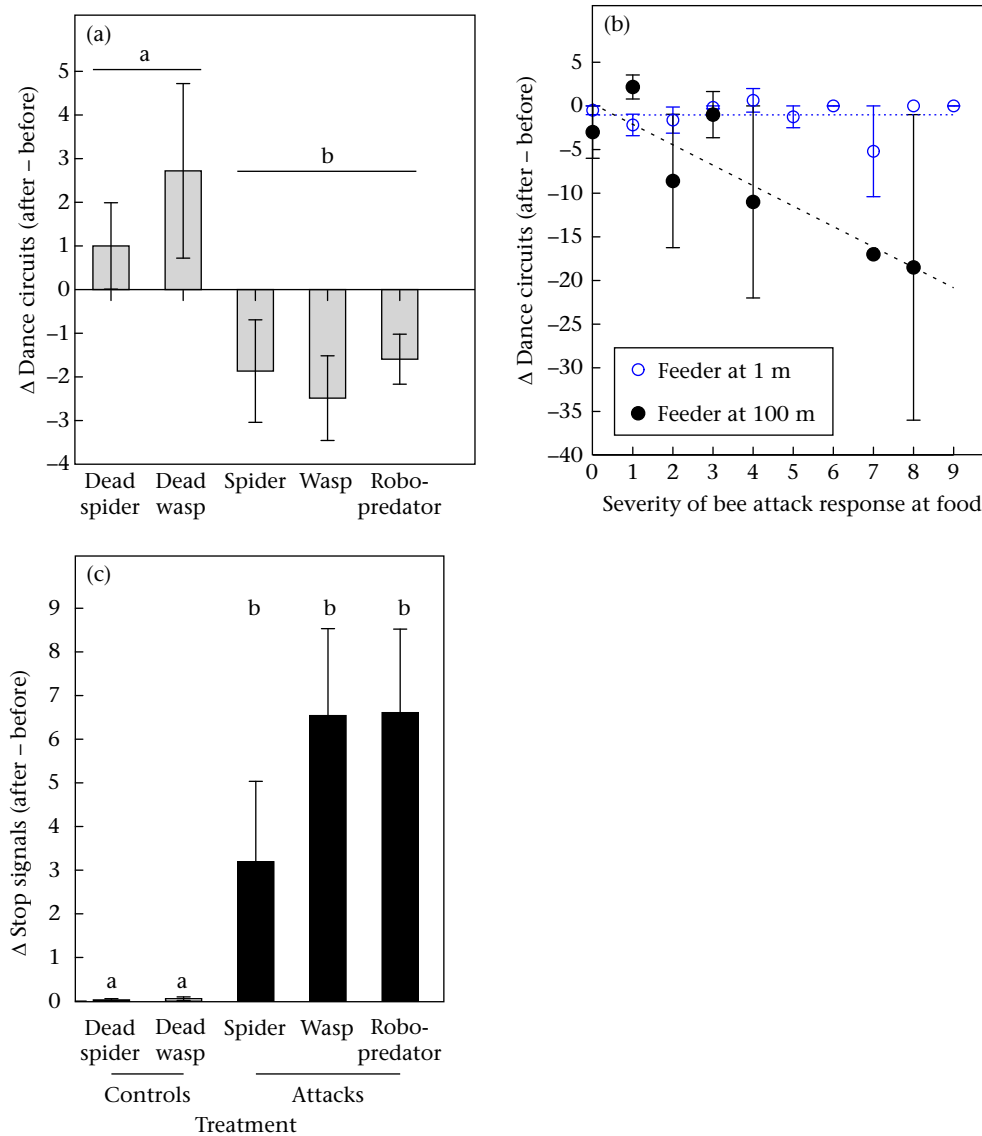


Figure 4. (a) Effect of predator attacks on bees' recruitment dancing for food (change in bee behaviour before versus after attacks), with significant differences between control and attack treatments shown by different letters. Data from all sucrose concentrations and distances were pooled because of a lack of sucrose and distance effects. (b) Relationships between the severity of bee responses on the feeder to attack and subsequent recruitment dancing at the different feeder distances (dashed linear regression lines shown). (c) Effect of predator attacks on stop signal production. Here, data from both feeder distances were pooled because there was no significant effect of feeder distance on stop signalling. Different letters indicate significant differences. Control treatments were a dead spider (*P. viridans*) or a dead wasp (*V. pensylvanica*). Attack treatments were a live spider (*P. viridans*), live wasp (*V. pensylvanica*) or the robo-predator. All plots show standard error bars.

predator attacks may elicit even more severe bee responses. Stop signalling was elicited to approximately the same degree by all types of attacks. There was no significant effect of predator type, sucrose concentration, food distance or bee response severity upon stop signal production. Thus, bee decisions to reduce their own recruitment dancing depended upon food distance and how severely they responded to attacks. Bee decisions about how much to inhibit dancing for the dangerous location simply depended upon their being attacked.

Effects on Recruitment Dancing

Honeybees are known to reduce recruitment dancing when returning from flowers containing recently killed bees (Abbott & Dukas, 2009) or after seeing or smelling a praying mantis predator on food (Bray & Nieh, 2014). However, it was not known

whether predation attempts by live predators affect recruitment dancing. We show that, as expected, live predator attacks can also reduce recruitment dancing. Unlike Bray and Nieh (2014), we did not observe decreased recruitment dancing in response to the dead predator controls (Fig. 4b). In addition, we did not observe foragers avoiding the dead predators, unlike other studies with dead spiders (Brechtbühl, Kropf, & Bacher, 2010; Dukas, 2001). In these studies (Brechtbühl et al., 2010; Dukas, 2001), bees approached a food source that already had a predator or a dead bee on it before they fed. However, in our experiment, dead predators were presented 3 mm behind the forager without making contact with bee, to match the live predator treatments. Bees may not have always seen or smelled the predator. Alternatively, they may have detected but ignored the predator. Once a honeybee begins feeding, it is highly focused on this task, and a sizeable disturbance is often required for it to stop feeding. Experimenters routinely take advantage of this

behaviour to apply multiple paint marks to the thoraces and abdomens of feeding bees (von Frisch, 1967).

We found a significant negative correlation between the severity of bee responses on the feeder to attack and the number of dance circuits subsequently produced by these bees at 100 m, but not at 1 m (Fig. 4b). Closer food sources reduce the time and energy costs of travel (Pyke, 1984), and bees generally judge nearer resources to be more valuable by performing more recruitment dances for closer as compared to farther food (Seeley, 1994; Seeley et al., 1991). Our results suggest that recruitment dancing decisions depend upon interactions between predation risk and cost of travel. However, honeybees typically forage over distances of more than 100 m (Couvillon et al., 2014) and future studies examining the effect of attacks at much greater distances would be beneficial.

The Benefits of Inhibitory Signals

There are multiple alternatives to using inhibitory signals, but our study illustrates a benefit: providing news. When foragers decrease excitatory recruitment signalling, recruitment declines. In our experiment, bees that were attacked produced significantly fewer recruitment dance circuits (Fig. 4a). However, bees that were not attacked continued to dance (Fig. 4a). Nieh (2010) showed that stop signallers preferentially targeted bees dancing for the food patch at which the stop signallers were attacked. Stop signals therefore enable foragers to inform nestmates of new information and more rapidly halt the recruitment process (Johnson & Nieh, 2010). An alternative to inhibitory signalling, competition between different positive processes, can also decrease recruitment to the losing process (Couzin, 2009). For example, different foragers in a honeybee colony can recruit for different food patches. A higher-quality patch will generate more recruitment dancing, leading to a decrease in foraging for the poorer site (Seeley et al., 1991). However, inhibitory signals should speed up abandonment of the poorer patch, a testable hypothesis. Finally, cues alone, information that has not been selected to convey information, can reduce an excitatory process. Foraging activity in harvester ants (*Pogonomyrmex barbatus*) is tuned to a cue, the rate at which patrolling ants return safely back to the nest (Greene & Gordon, 2007). *Lasius niger* ants also use a cue, increases in crowding, to inhibit subsequent trail pheromone deposition (Czaczkes, Grüter, Ellis, Wood, & Ratnieks, 2013). It would be interesting to model whether an inhibitory signal, like the no-entry pheromone signal of pharaoh ants (Robinson et al., 2005), would speed up both of these processes.

A Simple Rule?

We hypothesized that bees may modulate the level of inhibitory signalling according to food value (sucrose concentration and distance); that is, produce fewer inhibitory signals for higher-quality food. However, bees did not tune the level of stop signalling to resource sucrose concentration or distance. We also hypothesized that stop signallers would adjust their level of inhibitory signalling according to their experience of danger. Nieh (2010) showed that bees produced more stop signals in response to direct attack than to exposure to alarm pheromone, a second-hand indication of danger. However, more severe attacks did not significantly increase the number of stop signals produced. These results suggest a very simple rule: each bee produces stop signals if physically attacked, based upon some internal threshold, without modulating stop signalling according to the quality of the resource or the severity of the attack.

It is possible that more extreme changes in resource quality could affect individual stop signalling decisions. However, we chose a wide range of 0.5–2.5 M sucrose solution concentrations that

correspond to 16–66% sucrose (w/w) because generalist bee foragers collect nectar ranging from 10 to 70% sugar (Roubik et al., 1995). To facilitate dancing for these concentrations (1.0–2.5 M), we used a close range of distances (1 m and 100 m) that do not represent the full natural range of honeybee foraging. Thus, attacks on food at greater distances could increase the level of stop signalling as compared to food that is much closer. However, it is unclear whether such fine-tuning is necessary. As the distance to the food source increases, the level of recruitment dancing generally decreases (von Frisch, 1967), and thus there may be no need for a disproportionately greater inhibitory response.

Essentially, this lack of fine-scale stop signal modulation suggests that the decision about whether to stop recruiting is left to the recruitment dancers. These dancing bees modulate their dancing according to food quality and distance (Seeley et al., 2000) and are a primary regulatory agent of colony recruitment, with stop signals acting as a secondary agent that enhances system efficiency (Anderson & Ratnieks, 1999). Further fine-tuning of stop signalling is evidently not necessary. However, this lack of fine-tuning may not be a disadvantage given high variation in stop-signalling thresholds. The number of stop signals elicited even by a fixed stimulus such as the robo-predator (6.6 ± 15.9 stop signals elicited per bee) or exposure to alarm pheromone (Nieh, 2010) varies widely between bees. We found no evidence for individuals adjusting stop signalling according to how severely they responded to attacks on the feeder ($P = 0.34$). Even if such individual fine-tuning occurs, foraging is allocated and inhibited at the colony level, where the high variation between individuals would overwhelm putative individual tuning. This is not problematic because the colony can still maintain a finely tuned response given a diversity of individual signalling thresholds, as has been suggested for colony-level recruitment dancing (Mattila, Burke, & Seeley, 2008), shown for pollen and nectar foraging preferences (Page, 2013) and demonstrated theoretically for general division of labour (Bonabeau, Theraulaz, & Deneubourg, 1998).

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