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3 Honey bees tune excitatory and inhibitory recruitment signaling to resource value and predation risk

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21 **Abstract**

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

36

37 **Keywords:** inhibitory communication, negative feedback, foraging, recruitment, waggle dance, round dance, stop
38 signal, predation, danger

39

40 **Introduction**

41 Signals have been shaped through natural selection to convey information and, in many cases, can excite by
42 increasing actions or inhibit by decreasing actions. Signals that galvanize collective action for foraging are widespread
43 and can rapidly mobilize the group to exploit profitable food sources (Jeanson & Deneubourg, 2009). For example,
44 food-associated calls can increase foraging in chickens (C. S. Evans & Evans, 1999) and marmosets (Kitzmann &
45 Caine, 2009). In contrast, warning signals provide inhibition. Such signals are often triggered by predators (Blumstein,
46 1999; Căsar, Byrne, Young, & Zuberbühler, 2012; Clay, Smith, & Blumstein, 2012; Lemasson, Ouattara, Bouchet, &
47 Zuberbühler, 2010), and can inhibit foraging in a wide variety of birds and mammals (Caro, 2005).

48

49 In some cases, the excitatory and inhibitory signals are tightly coupled in a pair, and this close linkage improves
50 collective decision-making. The pharaoh ant uses a “no-entry” odor signal to indicate that a food odor trail is no
51 longer rewarding (Robinson, Jackson, Holcombe, & Ratnieks, 2005). A treehopper mother can reduce false alarms by
52 producing a vibrational signal that inhibits the alarm signal vibrations produced by its offspring (Hamel & Cocroft,
53 2012). Honey bees possess a powerful excitatory signal, recruitment dancing, which can rapidly increase the number
54 of workers visiting a resource (von Frisch, 1967). This recruitment signal is paired with a stop signal that inhibits
55 dancing (Kirchner, 1993; Nieh, 1993; Pastor & Seeley, 2005; Seeley et al., 2012) when foragers experience conspecific
56 attack (Nieh, 2010) or food source overcrowding (Lau & Nieh, 2009; Nieh, 1993; Thom, 2003). The stop signal is a
57 300-400 Hz vibrational signal with a duration of approximately 150 msec (Lau & Nieh, 2009; Seeley et al., 2012) that a
58 worker usually delivers while butting its head into the body of the receiver, causing the receiver to momentarily freeze
59 (Kietzman, 2015; Michelsen, Kirchner, & Lindauer, 1986; Nieh, 1993; Thom, Gilley, & Tautz, 2003). Although they
60 can be triggered by peril, stop signals are not necessarily warning signals because they inhibit recruitment dancing in
61 another context, house hunting (Seeley et al., 2012). In both contexts, foraging and house hunting, stop signals share a
62 common function. They increase the speed of colony decision-making by inhibiting recruitment.

63 Here, we examined the most common stop signal context, foraging, and determined the effect of predation and
64 food quality on stop signals. To date, no studies have demonstrated that predator encounters elicit stop signals,
65 although predation may be more common (Dukas, 2004; Morse, 1986) than conspecific fights over food or
66 overcrowding on a food source. Nieh (2010) elicited stop signaling when bees from different colonies fought for food,
67 smelled sting alarm pheromone, or were pinched with forceps. These latter two stimuli may also be experienced
68 during predator attacks, but attacks by real predators are necessary to demonstrate that predators can elicit stop signals.
69 In preliminary observations, we saw yellowjacket wasps (*Vespula pensylvanica*) and green lynx spiders (*Peucetia viridans*)
70 attacking foragers with varying degrees of severity, as gauged by predator behaviors and bee responses. We
71 hypothesized that foragers would produce stop signals and increase stop signal production in response to more severe
72 attacks from these predators.

73 The excitatory side of this signal pair, recruitment dancing, is strongly influenced by food value, and we therefore
74 wondered if stop signaling would also be affected by food quality. Bees perform more dance circuits and thereby
75 increase recruitment (Seeley, Camazine, & Sneyd, 1991) for higher-quality (sweeter) nectar (Seeley, Mikheyev, &
76 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 signaling and produce fewer stop signals after attacks at more profitable as compared to less profitable food.

Finally, recruitment dancing is influenced by predation. Honey bees 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 honey bee recruitment dancing (Nieh, 2010). However, it not clear if dancers adjust the level of recruitment dancing according to the severity of predator attack and if they weigh food profitability against attacks. If the food is sufficiently profitable, will non-lethal 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 honey bee recruitment dancing and stop signaling, an excitatory/inhibitory pair of signals. We sought to determine (1) if live predator attacks can elicit inhibitory signaling in bees, (2) if attack severity correlates with increased stop signaling and decreased recruitment dancing, and (3) if foragers consider food profit in modulating their level of excitatory or inhibitory signaling after predator attacks, discounting attacks if the food is very profitable.

Materials and Methods

Study site and colonies

We conducted our study at the UC San Diego Biological Field Station (BFS) in La Jolla, California from May to November of 2011. We used two 3-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 was 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 wore 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.

105

106 ***General methods***

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

113 To examine the effect of food profitability on stop signal production, we manipulated two variables: feeder
114 sucrose concentration and distance to the feeder. After training, we replaced the 2.0 M sucrose solution with a
115 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%,
116 and 65% sucrose (w/w) respectively. We chose these concentrations to represent a wide natural range of nectar sugar
117 concentrations. Generalist bee foragers, like honey bees, collect nectars ranging from 10–70% sugar (w/w) (Roubik,
118 Yanega, Aluja, Buchmann, & Inouye, 1995). Before measuring bee behavior, we allowed foragers to make five
119 collecting trips to adjust to the new sucrose concentration. Bees were trained to feeders 1 m or 100 m away from the
120 focal colony. Honey bees usually forage and recruit for natural food at much greater distances (Couvillon, Schürch, &
121 Ratnieks, 2014; Waddington, Herbert, Visscher, & Richter, 1994), but we chose these distances because 100 m was the
122 furthest distance to which we could reliably train bees for all the tested sucrose concentrations.

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

128 Experiments consisted of monitoring individual foragers before and after they received a treatment (Nieh,
129 2010). In the *before* phase, we randomly selected a forager that had just returned to the hive. A hive visit began when a
130 forager first walked onto the comb above the hive entrance and ended when it exited the hive through the hive
131 entrance or by flying away. Once the bee returned to the feeder, we applied a treatment: attack by a (1) live spider, (2)
132 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 behavior and sounds with a video camera (Sony HD-HC7, New York, USA). We manually tracked the focal bee with a microphone (Radio Shack model #33-3013 Fort Worth, Texas, USA) 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 #132170 Burbank, California, USA) 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 behavior, a signaler 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 the western yellowjacket (*V. pensylvanica*) attacking honey bees on feeders. *Peucetia viridans* (Oxyopidae) occurs throughout the southern United States, Mexico, and Central America, and often preys upon pollinators, including honey bees by waiting on inflorescences (Louda, 1982; Weems & Whitcomb, 2001). *Vespula pensylvanica* occurs naturally west of the Rocky Mountains from southern California to southwestern Canada (Visscher & Vetter, 2003) and can attack and capture live honey bees (Wilson & Holway, 2010). During preliminary observations, we observed 20 *V. pensylvanica* wasps performing 116 attacks on honey bees at feeders. These attacks ranged in severity from touching to grabbing and biting (4 trials, 2-hr total observation time). We therefore used these species as a predator.

Plastic vials were used to capture spiders on flowers and insect nets to capture wasps at the nest entrances of a separate set of honey bee 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 cm x 0.5 cm paper tether to each wasp thorax

161 and spider abdomen (Fig. 1A,B). To immobilize the wasps for tethering, we chilled them on ice for approximately 2
162 min. Spiders did not survive chilling, so we placed a spider in a small cup, applied the glue to the paper tether and
163 used forceps to quickly lower the tether onto its abdomen while it was moving. Compensating for the spider's motion
164 was tricky but possible with practice. After tethering, wasps and spiders were allowed to recover in a container for 30
165 min before use. On a given day, we used a different individual predator for each attack, and then returned the
166 predator to a holding container for the next experimental day. Each predator was used approximately twice. In the
167 holding container, wasps were given 1.0 M sugar solution, but spiders were not fed. Control spiders and wasps
168 consisted of dead specimens that we collected separately. We froze these live controls for approximately 10 min
169 before control trials to preserve their odor and appearance and then tethered them. To avoid having odors like honey
170 bee alarm pheromone on the predators, we did not expose these control predators to honey bees before using them
171 and used each control only once.

172 We used locking forceps to present the spiders and wasps. Once the focal bee landed, we approached it on its
173 posterior right side with a predator and maintained an initial separation of approximately 3 mm. We allowed the living
174 predator to initiate contact with the bee. An attack was defined as a predator contacting the bee. We chose an attack
175 duration of 10 s, defined as the time that a predator contacted a bee, because this was the approximate duration of
176 natural *V. pensylvanica* attacks on honey bees at a feeder at our field site (pers. obs.). If a bee moved to a new position
177 on the feeder or flew away and subsequently returned, we again placed the predator 3 mm away from the bee and
178 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
179 a 10 s forager attack. Predators usually began an attack within a few seconds of being placed close to the focal bee.

180 Honey bees that stung subsequently died, and thus we did not use data from bees that stung predators
181 because these bees did not return to the nest to perform recruitment dances or produce stop signals. We therefore
182 tried to avoid fatal attacks and pulled the predator back if a deadly encounter was about to occur: a bee about to sting
183 the predator, a wasp about to sting the bee, or a spider about to bite the bee. This method of preventing fatal attacks
184 generally worked: only 3% of bees died.

185 One feeder observer provided the predator treatment and a second one recorded the number and type of
186 predator attacks and the bee's responses at the feeder. During control trials, we placed a freshly dead predator at
187 ambient air temperature also approximately 3 mm on the posterior right side of bee. Bees sometimes turned to face

the predator and could therefore 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 behavior 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 behaviors. *Touching* consisted of making a brief contact, usually <2 s. *Grabbing* was the predator hooking a leg onto bee and drawing it in. *Biting* was making contact with mouthparts, but not necessarily piercing the cuticle, which could be fatal with a spider predator. Touching was likely an attack behavior, not simply the result of the predator attempting to escape, because touching was always subsequently followed by at least one other clear attack behavior (grabbing or biting). These attack behaviors are similar to those observed by Rangel et al. (2010) between honey bee workers freely fighting over a nest site. Although predators were tethered in our trials, they exhibited similar attack behaviors to un-tethered 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 objectively consider these attacks, *we defined attack severity as the sum of all predator attacks during an attack bout*. For instance, two attacks during an attack bout results in 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 bee responses to attack. *Moving away* consisted of a bee walking to a different part of the feeder. *Flying away* was a bee flying away. *Grappling* was a bee fighting back and using its legs to hold onto the predator. *Attempting to sting* consisted of a bee arching its abdomen and pressing 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 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 electro-mechanical CKD AS-0RN 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. 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 center 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, Oregon, USA). To determine the actual force levels, we modified the procedure of Tautz et al. (1995). We measured force with a type 8001 impedance head attached to a type 4294 vibrational calibrator (Brüel & Kjaer, Norcross, Georgia, USA). The impedance head was pressed against the center 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 honey bee 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 >97% of bees. No bees 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 odors.

Statistics

All data were analyzed using JMP v9 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 wasps, 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 use log-transformed $\#dance\ circuits_{before\ phase}$. We tested the effect of treatments on $\log\Delta dance\ circuits = \#dance\ circuits_{after\ phase} - \#dance\ circuits_{before\ phase}$. 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 $\Delta stop\ signals = \#stop\ signals_{after\ phase} - \#stop\ signals_{before\ phase}$. 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 non-significant (NS, $P < 0.05$). Averages are reported as mean $\pm$ 1 standard deviation.

Results

We tested 259 bees from two colonies (112 and 147 bees from colonies 1 and 2, respectively). 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 treatments and 14.1 ± 20.1 s after treatments.

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 (NS), and colony accounted for 21% of model variance.

Wasp and spider attacks altered bee behaviors 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, biting only accounted for 3.5% of live predator attack behaviors ($n=314$ total attack behaviors), 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).

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). However, 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 NS, and colony accounted for <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 bee flying away in response to more severe attacks was strongly influenced by a single outlier point 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 (NS). Colony accounted for <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 4 dance circuits per performance (Fig. 4A, predator type effect: $F_{1,250}=2.56$, $P=0.039$). 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, and robo-spider) as compared to controls (dead spider and dead wasp, contrast test: $F_{1,252}=6.28$, $P=0.01$). All interactions were *NS* and colony accounted for <1% of model variance.

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 *NS*, colony accounted for <1% of model variance). At 1 m, the effects of bee response severity, sucrose, and the interaction were all *NS*.

Predator attacks increased stop signal production

Predator attacks increased stop signaling 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 treatments (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 *NS* and colony accounted for 11% of model variance.

Discussion

The honey bee 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 easily 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 the live predators or the robo-predator or produced significantly fewer stop signals than bees exposed to dead predators, which did not attack. These data provide the first demonstration that decreases in honey bee 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 predator attacks may elicit even more severe bee responses. Stop signaling 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

Honey bees 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 if 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 wasp or dead spider 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 seen or smelled the predator. Alternatively, they may have detected but ignored the predator. Once a honey bee 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 behavior 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, honey bees typically forage over distances >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 signaling, recruitment will decline. 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 signalers preferentially targeted bees dancing for the food patch at which the stop signalers were attacked. Stop signals therefore enable foragers to inform nestmates of new information and more rapidly halt the recruitment process (Johnson & Nieh, 2010). Another alternative to inhibitory signaling, competition between different positive processes, can also decrease recruitment to the losing process (Couzin, 2009). For example, different foragers in a honey bee 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 if an inhibitory signal, like the no-entry pheromone signal of pharaoh ants (Robinson et al., 2005), would speed up both of these processes.

381 *A simple rule?*

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

391 It is possible that more extreme changes in resource quality could affect individual stop signaling decisions.
392 However, we chose a wide range of 0.5-2.5 M sucrose solution concentrations that correspond to 16-66% sucrose
393 (w/w) because generalist bee foragers collect nectar ranging from 10-70% sugar (Roubik et al., 1995). To facilitate
394 dancing for these concentrations (1.0-2.5 M), we used a close range of distances (1 and 100 m) that do not represent
395 the full natural range of honey bee foraging. Thus, attacks on food at greater distances could increase the level of stop
396 signaling as compared to food that is much closer. However, it is unclear if such fine-tuning is necessary. As the
397 distance to the food source increases, the level of recruitment dancing generally decreases (von Frisch, 1967), and thus
398 there may be no need for a disproportionately greater inhibitory response.

399 Essentially, this lack of fine-scale stop signal modulation suggests that the decision about whether to stop
400 recruiting is left to the recruitment dancers. These dancing bees modulate their dancing according to food quality and
401 distance (Seeley et al., 2000) and are a primary regulatory agent of colony recruitment, with stop signals acting as a
402 secondary agent that enhance system efficiency (Anderson & Ratnieks, 1999). Further fine-tuning of stop signaling is
403 evidently not necessary. However, this lack of fine-tuning may not be a disadvantage given high variation in stop
404 signaling thresholds. The number of stop signals elicited even by a fixed stimulus such as the robo-predator (6.6 ± 15.9
405 stop signals elicited per bee) or exposure to alarm pheromone (Nieh, 2010) varies widely between bees. We found no
406 evidence for individuals adjusting stop signaling according to how severely they responded to attacks on the feeder
407 ($P=0.34$). Even if such individual fine-tuning occurs, foraging is allocated and inhibited at the colony level, where the
408 high variation between individuals would functionally overwhelm putative individual tuning. This is not problematic

because the colony can still maintain a finely tuned response given a diversity of individual signaling 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 labor (Bonabeau, Theraulaz, & Deneubourg, 1998).

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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 part C) prevented the armature from extending too far.

547 **Figure 2.** Effect of food quality (sucrose concentration) on recruitment dancing. Mean, standard error bars, and linear
 548 regression line shown. Foragers collected, but did not dance for, 0.5 M sucrose solution.
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556 **Figure 3.** Responses of bees on the food source to live predator attacks. A) Distribution of responses to attackers
 557 (standard error bars shown). B) Total bee responses per attack at different attack severity levels by spiders and wasps.
 558 The effect of attack severity by (C) spiders and (D) wasps on the most common bee response, flying away. Linear
 559 regression lines and equations are shown.
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563 **Figure 4.** Effect of predator attacks on (A) recruitment dancing for food with significant differences between the
564 control and attack treatments shown by different letters. Data from all sucrose concentrations and distances were
565 pooled because of a lack of sucrose and distance effects. (B) The relationships between the severity of bee responses
566 on the feeder to attack and subsequent recruitment dancing are given at the different feeder distances (dashed linear
567 regression lines shown). (C) The effect of predator attacks on stop signal production. Here, data from both feeder
568 distances were pooled because there was no significant effect of feeder distance on stop signaling. Different letters
569 indicate significant differences. Control treatments were a dead spider (*P. viridans*) or a dead wasp (*V. pensylvanica*)
570 Attack treatments were a live spider (*P. viridans*), live wasp (*V. pensylvanica*), or the robo-predator. All plots show
571 standard error bars.
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