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Report

A Negative Feedback Signal That Is Triggered by Peril Curbs Honey Bee Recruitment

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Summary

Decision making in superorganisms such as honey bee colonies often uses self-organizing behaviors, feedback loops that allow the colony to gather information from multiple individuals and achieve reliable and agile solutions. Honey bees use positive feedback from the waggle dance to allocate colony foraging effort. However, the use of negative feedback signals by superorganisms is poorly understood. I show that conspecific attacks at a food source lead to the production of stop signals, communication that was known to reduce waggle dancing and recruitment but lacked a clear natural trigger. Signalers preferentially targeted nestmates visiting the same food source, on the basis of its odor. During aggressive food competition, attack victims increased signal production by 43 fold. Foragers that attacked competitors or experienced no aggression did not alter signal production. Biting ambush predators also attack foragers at flowers. Simulated biting of foragers or exposure to bee alarm pheromone also elicited signaling (88-fold and 14-fold increases, respectively). This provides the first clear evidence of a negative feedback signal elicited by foraging peril to counteract the positive feedback of the waggle dance. As in intra- and intercellular communication, negative feedback may play an important, though currently underappreciated, role in self-organizing behaviors within superorganisms.

Results

Cycles of positive and negative feedback are key elements of information processing in all biological systems. Such feedback cycles improve information flow and decision making at multiple levels, including intra- and intercellular signaling [1]. In superorganisms, individuals within a social group act as cooperative vehicles for gene propagation, and their actions often rely on a network of self-organizing behaviors, rather than centralized control [2]. These behaviors use a series of simple, repeating feedback loops [3] that have largely been modeled as positive feedback cycles. These cycles allow a colony to benefit from the information of multiple individuals. Collective decision making allows such multiple processing units (information receivers) to arrive at reliable and robust solutions [4, 5]. Group decision making in tasks such as house hunting [6, 7], nest organization, and foraging provide classic examples [8]. The role of self-organizing feedback loops has been particularly well explored in foraging, which is frequent and plays a crucial role in colony fitness. Bumble bees (*Bombus terrestris*) returning from a rich food source can produce a foraging activation pheromone [9]. Honey bees (*Apis mellifera*) waggle dance to recruit nestmates to resources such as food, water, and resin [10]. In both cases, individuals generate positive feedback recruitment signals based on internal response thresholds, and allocation of the foraging force results from the sum of individual signalers [11].

However, relatively little is known about the role of negative feedback signals in superorganism behavior [12]. The clearest example is the Pharaoh's ant (*Monomorium pharaonis*), which deposits recruitment pheromone that generates positive feedback but can also use a negative, repellent pheromone to mark unrewarding odor trails and thus prevent the system from being caught in a suboptimal solution [13, 14]. In honey bees, the waggle dance is a powerful source of positive feedback that can rapidly increase foraging at a specific location, providing significant fitness benefits for the colony [15, 16]. However, there is a signal, which remains poorly understood, that evidently counteracts the positive feedback provided by the waggle dance.

The stop signal is a brief vibrational signal lasting 150 ms [17] at around 380 Hz [18]. It is frequently delivered by a sender butting her head into a recipient, although the sender may also climb on top of the receiver [19]. Occasionally, the signal is delivered to the comb [19, 20], but most signals are received by waggle dancers [20]. The stop signal was originally called a "begging call," because the signaler was thought to obtain a food sample from the receiver [10, 21]. However, stop signals do not elicit food exchange [20, 22]. It has also been called the "brief piping signal" because its dominant frequency is similar to other worker piping signals [19, 23]. I will use the term "stop signal" because experiments show that this signal can cause waggle dancers to stop dancing and leave the nest [17, 20, 22]. Playbacks of the stop signal (artificial vibrations of the comb) reduced waggle dance durations by 59% and recruitment by 60% [17]. Natural and synthesized signals (but not white noise) significantly increased waggle dancer departure when delivered directly to dancers through a vibrating rod [20]. Both of these studies used artificial food sources. Pastor and Seeley [22] studied bees foraging at natural floral resources and found that recipients of natural stop signals ceased waggle dancing significantly more often than expected by chance alone.

Why do honey bees need a negative feedback signal to inhibit foraging? Perhaps one key to this mystery lies in the observation that deteriorating foraging conditions increase stop signal production. Thom et al. [19] reported that stop signal production increased at a crowded feeder and suggested that scramble competition could elicit signals. Recently, Lau and Nieh [24] found that feeder foragers received more stop signals when they experienced a longer wait time to feed at a crowded as compared to an uncrowded feeder. Thus, the stop signal may be triggered by a variety of conditions linked to declining resource profitability. If so, signalers should target nestmates visiting the same resource, because recruitment should not be stopped for all resources. I tested this prediction by training foragers to two different feeders and determining whether signalers preferentially signaled bees from their own feeder.

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My preliminary observations suggested that conspecific fighting over rich food increased stop signal production. Such fighting could occur in the context of nest robbing [25–27] but is probably not common for floral resources. Bees must generally visit multiple flowers scattered throughout a patch to collect a full nectar load [26]. Such dispersed flowers, each offering only a small reward, would probably not favor aggressive monopolization. However, honey bees can evidently produce stop signals after returning from floral resources [22]. What are they communicating? Honey bees are attacked on flowers by ambush predators such as praying mantids (Mantidae [28]), predacious bugs (Hemiptera [29]), some social wasps [30], and crab spiders [31]. Crab spiders maximized prey encounters by spending less time hunting on old flowers than on new flowers that provide more nectar [32]. Morse [33] also reported that honey bees had a daily 9.2% probability of being attacked by a spider (3% probability of capture) while foraging on milkweed. In fact, Dukas [34] suggested that honey bees may reduce recruitment to a specific food patch when they encounter predators. Predator attacks may be a natural trigger for stop signals. I therefore decided to test whether the main stimuli associated with attack (biting and alarm pheromone release) would increase stop signal production.

Stop Signal Specificity Experiments

In the different-odor experiment, signalers significantly targeted foragers visiting the same location, delivering (on average) five times more signals to foragers from the same feeder than to foragers visiting the other feeder (receiver type: $F_{2,164} = 13.08$, $p < 0.0001$). The sender's feeder location did not affect targeting specificity (sender feeder location: $F_{1,164} = 0.08$, $p = 0.78$). Signalers targeted bees visiting the same location (Figure 1A). The interaction of receiver type and sender feeder location was not significant ($F_{2,162} = 2.37$, $p = 0.10$).

When both feeders had the same odor, there was no targeting among feeder bees. Same- and different-feeder bees received approximately equal numbers of signals (Figure 1B). In this same-odor experiment, there is no effect of receiver type ($F_{2,61} = 1.23$, $p = 0.30$), feeder location ($F_{1,61} = 0.36$, $p = 0.50$), odor type ($F_{1,61} = 2.88$, $p = 0.09$), or any interactions ($F_{2,54} \leq 2.08$, $p \geq 0.13$). Thus, signal targeting can be abolished by providing the same strong scent at both locations (Figure 1B). Foragers also signaled nestmates that were not foragers ("other" bees). However, they signaled "other" bees significantly less than expected: the number of signals delivered to each receiver type was different from random (different-odor experiment, $\chi_2^2 = 965.4$, $p << 0.0001$; same-odor experiment, $\chi_2^2 = 2331.1$, $p << 0.0001$; Figure 1B, observed and expected signals).

Competition Experiment

All attacks were between competitors and resident foragers. Fights consisted of one individual (attacker) biting another individual (victim) on the legs, wings, abdomen, or head for 1.4 ± 1.5 s, primarily in the first hour of competition. No attacks were mortal, although 6% resulted in prolonged grappling. Residents continued visiting the feeder throughout the competition phase, but reduced recruitment (Figure 2A). At the same time, the number of stop signals (measured as the total number of signals produced and received by a focal forager during each nest hive visit) increased ($F_{1,19} = 12.0$, $p = 0.003$, Figure 2B). Focal foragers received over 90% of these signals (n = 345). After 80 min, foragers ceased fighting and focused on food collection, although invaders and residents avoided contact with each other on the feeder.

When bees fed *undisturbed* in the presence of competing bees, they exhibited no change in measured behaviors (Figure 3). There was no change in stop signal production (no signals), the number of waggle circuits ($W_{20} = 16.0$, $p = 0.17$),

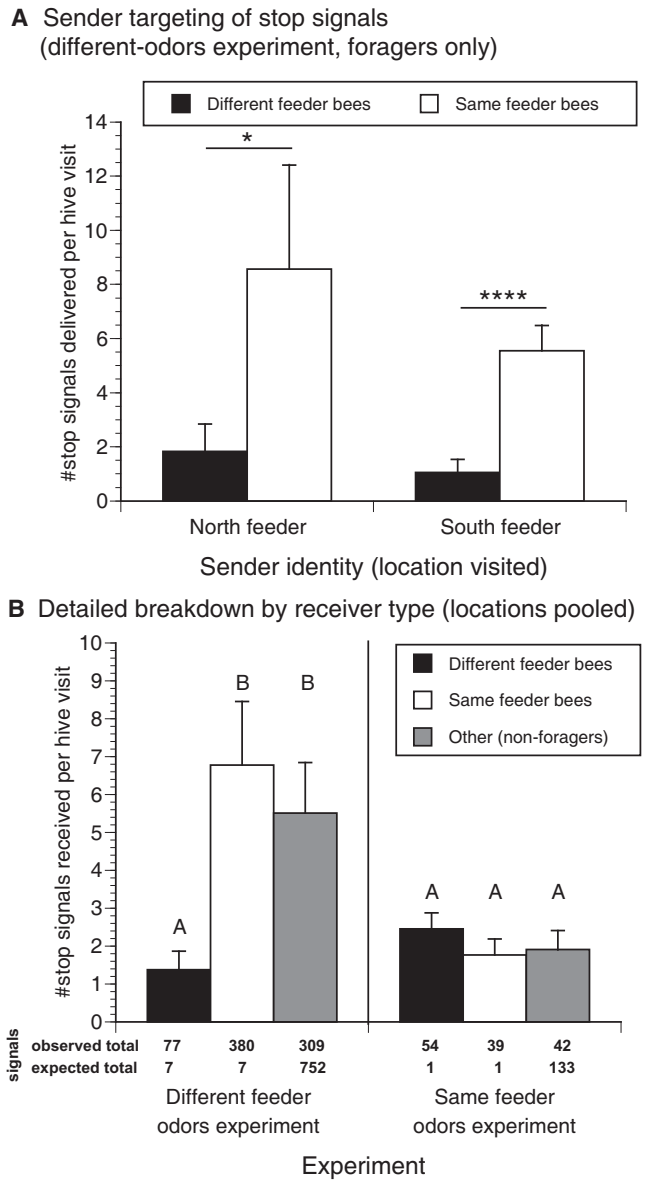

Figure 1. Results of the Stop Signal Specificity Experiment

(A) Targeting among foragers (34 trials). Horizontal lines with stars indicate significant differences (north senders: $F_{1,43} = 7.07$, *p = 0.011; south senders: $F_{1,63} = 50.23$, ****p < 0.0001).

(B) Distribution of stop signals among all receiver types (different-odor treatment: locations have different odors; same-odor treatment: locations have same odor). Other bees are nestmates that received stop signals but did not visit either feeder and are not active foragers. Data from north and south senders are pooled because there is no significant effect of location. Different letters above each bar indicate significant differences (Tukey HSD, $\alpha = 0.05$, $Q = 2.365$, *p < 0.05).

White bars: signals to bees visiting same feeder as sender. Black bars: bees visiting different feeder from sender. Mean ± 1 standard error (SE) is shown.

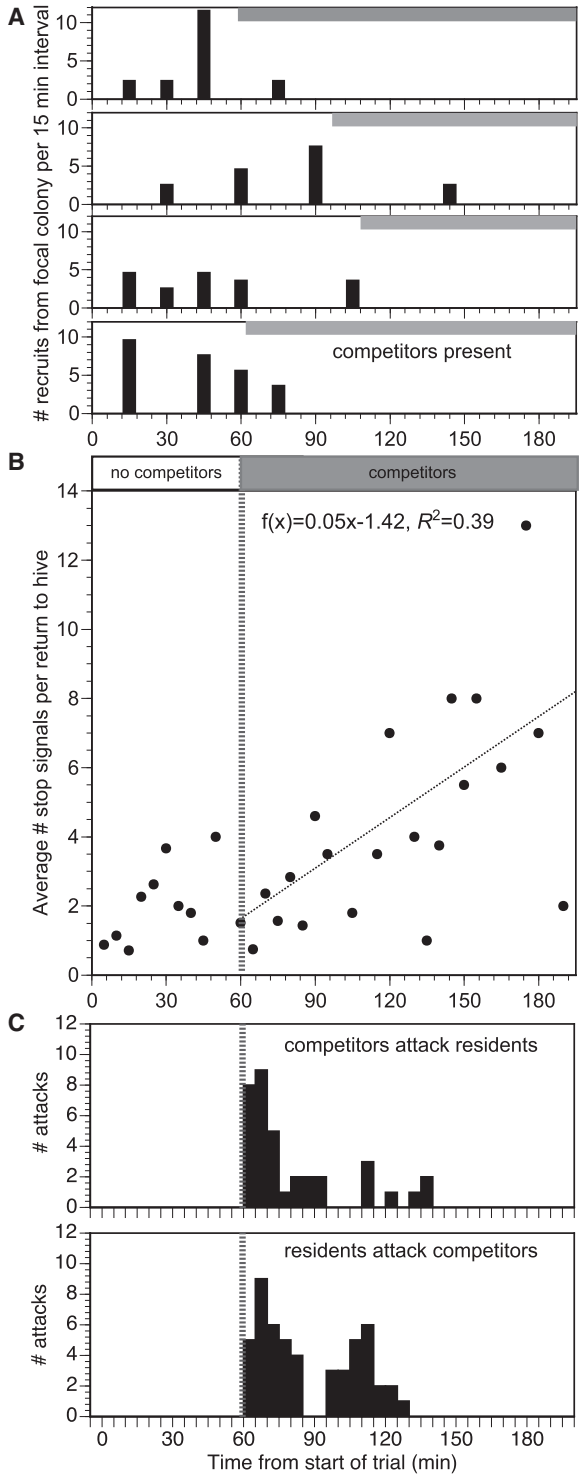

Figure 2. Competition Experiment: Effect of Aggressive Competition at the Resource

Effect of competition on (A) recruitment (four representative trials shown), (B) stop signal production (linear regression equation and line for the competition phase shown, p = 0.003), and (C) fighting.

hive visit duration (W_{20} = 26.0, p = 0.35), food unloading wait time (33.24 ± 33.70 s, W_{20} = 9.5, p = 0.74), or tremble dancing (no trembling). Bees that *attacked* competitors also did not change their behavior (Figure 3). There was no change in stop signal production (W_{20} = 1.5, p = 0.50), the number of waggle circuits (W_{20} = 26.5, p = 0.14), hive visit duration (W_{20} = 50.0, p = 0.06), food unloading wait time (36.60 ± 33.22 s, W_{20} = −41.0, p = 0.13), or tremble dancing (W_{20} = 1.0, p = 0.99).

However, bees that were *victims* of attack produced significantly more stop signals (W_{20} = 105.0, p < 0.0001), increasing average signal production by 43 fold (Figure 3). Victims significantly decreased waggle dancing by 12.6 fold (W_{20} = −50.0, p = 0.002). Tremble dancing significantly increased (W_{20} = 14.0, p = 0.016) from zero to an average 35% of hive visits with tremble dancing. Hive visit duration (W_{20} = −2.0, p = 0.96) and food unloading wait time (27.39 ± 21.49 s, W_{20} = 31.5, p = 0.25) were unaffected. Thus, only victims significantly altered their nest behavior. They produced more stop signals, increased tremble dancing, and decreased recruitment (produced fewer waggle circuits).

Physical Aggression Experiment

Bees responded similarly to conspecific attacks and pinching. Victims struggled to escape and occasionally produced alarm pheromone. After pinching, foragers generally resumed sugar solution collection. There was a strong and significant effect of physical aggression (pinching) on stop signal production (W_{20} = 63.0, p = 0.0003, Figure 4). The average number of stop signals produced per hive visit increased to the highest levels recorded in any experiment (88-fold increase). Bees sharply decreased waggle dancing by 278 fold (average number of waggle circuits per hive visit is 13.9 before and 0.05 after pinching, W_{20} = −68.0, p < 0.0001). Hive visit duration increased 4 fold (W_{20} = 78.0, p = 0.0008). Pinched foragers unloaded and then walked around the dance floor before leaving the nest. These bees experienced the same unloading wait times before and after pinching (31.01 ± 42.89 s, W_{20} = −9.0, p = 0.75) and did not change levels of tremble dancing (W_{20} = 15.0, p = 0.20). Thus, pinched foragers behaved much like naturally attacked bees. They increased stop signaling and decreased waggle dancing.

Gland Extract Experiment

Foragers showed no response to mandibular gland extract or to control (hexane-only) treatment. They did not move away or stop feeding. There was no significant effect of *mandibular* gland extract on stop signal production (W_{20} = 1.0, p = 0.99), the number of waggle circuits (W_{20} = −4.5, p = 0.72), hive visit duration (W_{20} = −2.0, p = 0.95), food unloading wait time (W_{20} = 25.0, p = 0.37), or tremble dancing (W_{20} = 3.5, p = 0.44, Figure 4).

Foragers exhibited an immediate alarm response to sting gland extract, walking away and sometimes leaving the feeder during exposure. There was strong and significant effect of sting gland extract on stop signaling (Figure 4). Sting gland extract increased the average number of stop signals by 14 fold (W_{20} = 49.5, p = 0.003). No other behaviors were affected: waggle dancing (W_{20} = −30.0, p = 0.034, NS_{SB}), tremble dancing (W_{20} = 3.5, p = 0.44), unloading wait time (W_{20} = −50.5, p = 0.04, NS_{SB}), or hive visit duration (W_{20} = 23.0, p = 0.41).

Discussion

These experiments provide the first evidence that forager peril can elicit a negative feedback signal to counter the honey bee waggle dance, providing a crucial element in the feedback loops that control decisions in a self-organizing

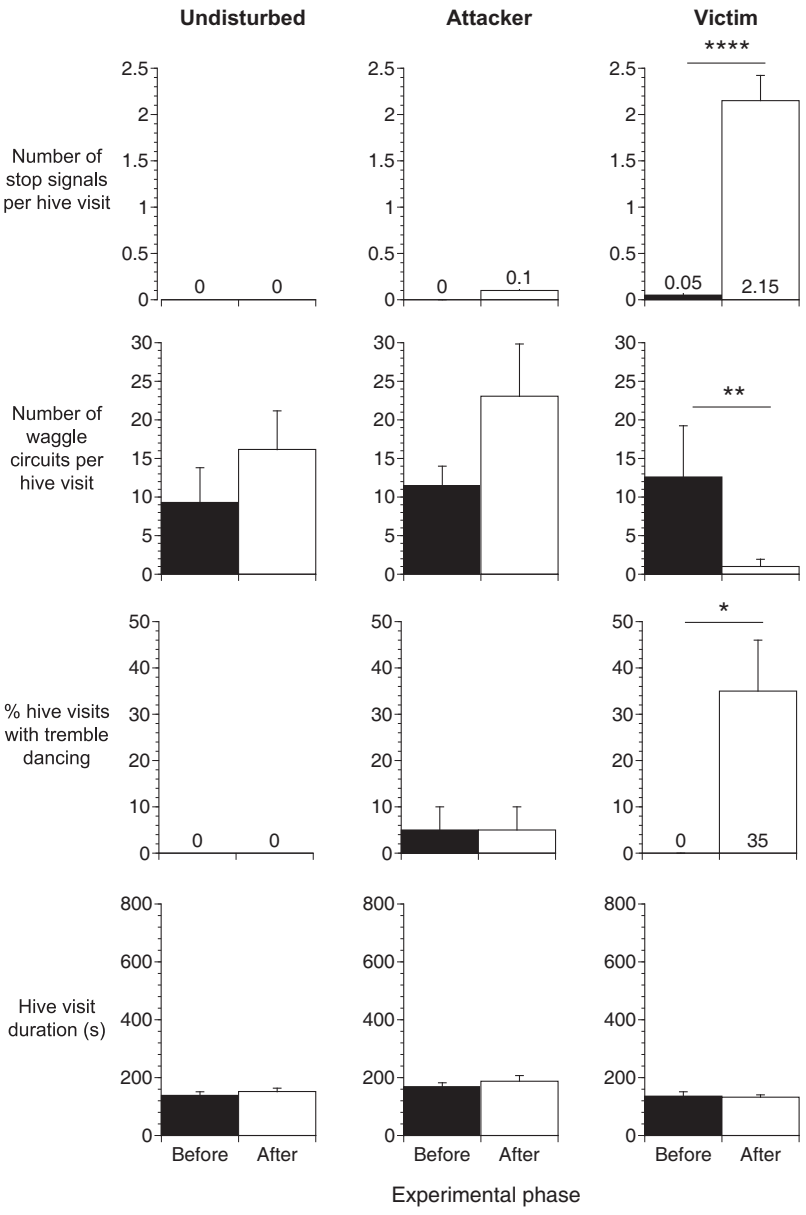

Figure 3. Competition Experiment: Changes in Forager Intranidal Behavior before and after Competition

Changes in forager intranidal behavior before (black bars) and after (white bars) competition (mean ± 1 SE) are shown. In the competition phase, foragers received and delivered no aggression (undisturbed), attacked a competitor (attacker), or were attacked by a competitor (victim). The after phase shows their subsequent behavior during their first trip back to the nest. Horizontal lines with stars indicate significant differences (*$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$).

superorganism. During competition for a rich food source, feral bee competitors attacked resident bees. Bees that were attacked (victims) increased the number of stop signals by 43 fold, began to tremble dance, and sharply decreased (by 12.6 fold) the number of waggle dance circuits performed. Bees that were undisturbed (received and gave no attacks) and bees that attacked competitors continued to recruit and produced almost no stop signals (Figure 3). Senders targeted bees that smelled like the location visited (Figure 1). The proximate causes of stop signal production can be further parsed into receiving physical aggression (biting) and detecting alarm pheromone. Pinching a bee or exposing it to alarm pheromone is sufficient to elicit an 88- or 14-fold increase, respectively, in stop signal production (Figure 4). Thus, physical attack or alarm pheromone exposure is sufficient to trigger signal production, stimuli also elicited by ambush predators on floral resources.

The self-organizing nature of this signal is exemplified by how receivers responded. Stop signals reduce recruitment, causing waggle dancers to prematurely end their dancing [17, 20, 22]. This is a modulatory process in which an accumulation of signals, generally from multiple signalers, increases the probability that waggle dancers will cease recruiting [20]. Signalers (victims) directed most signals at foragers visiting the same patch (Figure 1A). Signal receivers decreased recruitment (Figure 2A). As more bees became victims of attack, the total number of stop signals increased and recruitment ceased (Figure 2).

Stop Signal Specificity

Each forager used odor at the food source as a template to recognize nestmates visiting the same location (Figure 1). This could be problematic because colonies can recruit for the same floral species at multiple locations [26]. However, foragers can carry the odor of a floral species and strong odors associated with a given location [10]. Thus, foragers could distinguish nestmates visiting different locations if floral or location odors varied sufficiently.

Overall, signal receivers visiting the same-scented location were 0.1% of bees on the dance floor, yet received 50%–69% of all signals, an impressive degree of targeting. However, it is unclear why "other" bees also received signals (31% same- and 40% different-feeder odor experiment). Such signals could be errors and might occur when (1) bees transfer their location-acquired odor to nestmates (quite possible given the very high scent levels applied in our experiment), (2) the sender lunges to signal a same-scented bee but misses and signals a different bee, (3) there is imperfect template matching (the sender's rules and its sensory perception operate with less than perfect accuracy), or all three. It would be informative to determine whether receiver responses vary with the signaler's odor. The appropriateness of responses would improve if receivers pay more attention to signalers visiting the same location. Stop signals could also provide a different message to this "other" category of bees, perhaps enhancing the labor reallocation message of the tremble dance [35], as suggested by Thom et al. [19].

Imperfect targeting accuracy may be sufficient for a modulatory signaling system. Stop signals modulate and significantly increase the probability of waggle dancers leaving the nest, but dancers do not generally show an immediate response to a stop signal [20, 22]. Receivers requiring multiple stop

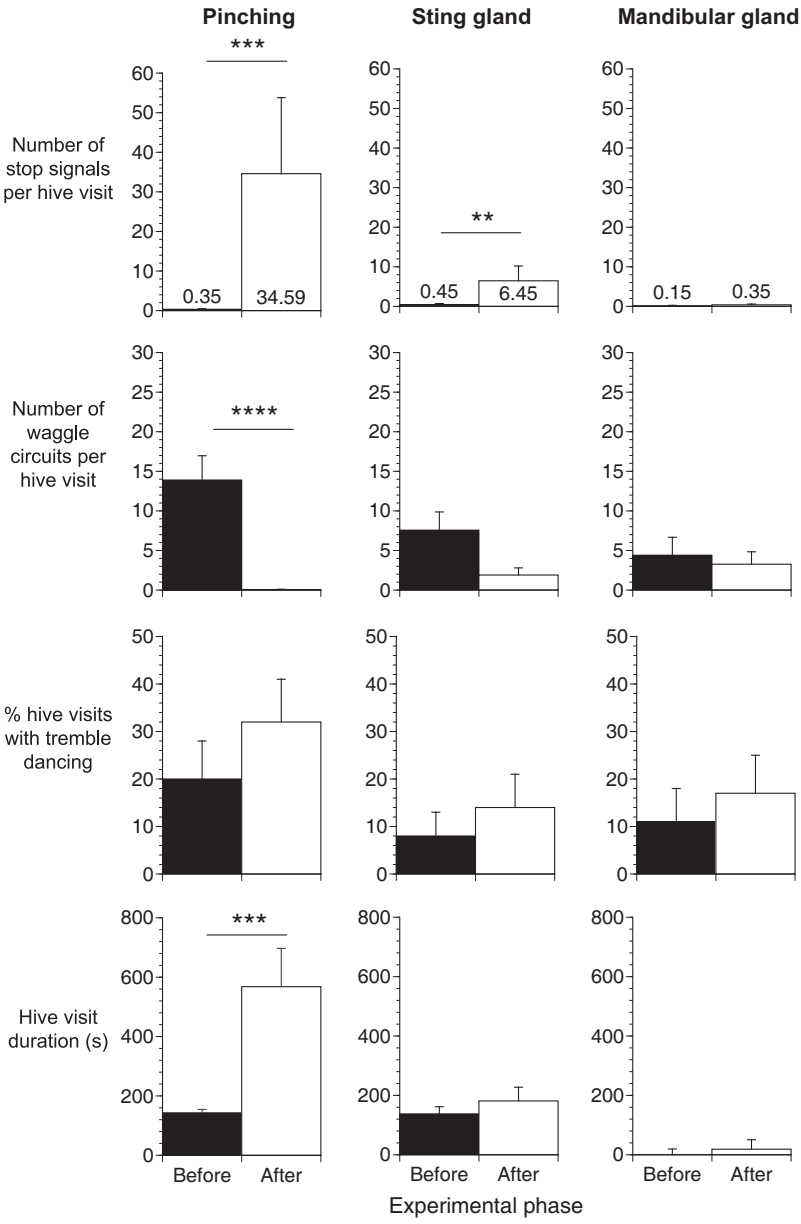

Figure 4. Changes in Forager Intranidal Behavior before (black bars) and after (white bars) Pinching Attacks and Gland Extract Exposure

In these experiments, there were no competing bees. In the treatment phase, foragers received a pinch or were exposed to gland extracts. The after phase shows their subsequent behavior during their first trip back to the nest. The average (±1 SE) number of stop signals per hive visit is given because many values are quite low. Horizontal lines with stars indicate significant differences (**p < 0.01, ***p < 0.001, ****p < 0.0001). Black bars are before and white bars are after pinching attacks and gland extract exposure.

signals are, in effect, integrating negative feedback from multiple information sources, and the colony-wide effect of recruitment cessation (Figure 2A) thus arises as an emergent property of multiple, independent actors signaling and receiving information about food patch conditions [4].

Proximate Stimuli

As in natural aggression (Figure 3), pinching a forager's leg or exposing a forager to alarm pheromone sharply increased stop signal production. Pinching led to a 6-fold signaling increase relative to alarm pheromone alone (Figure 4), perhaps because pinching sometimes resulted in alarm pheromone release (providing dual danger-associated stimuli). Like a natural attack, pinching also sharply decreased the number of waggle dance circuits. Alarm pheromone did not affect waggle dancing production, although there was a 4-fold decrease in the average number of waggle circuits. Thus, more dangerous attack stimuli appear to elicit stronger responses (more stop signals, fewer waggle dance circuits) as compared to alarm pheromone alone, which involved no physical contact.

The role of mandibular gland secretions in foraging is unclear. However, these secretions did not affect stop signal production (Figure 4). Worker mandibular gland extract elicited no aggression, attraction, or repulsion from guard bees at the nest [36]. However, when 2-heptanone, a major component of worker mandibular glands, was applied on flowers, it exerted a repellent effect [36]. I found no aggression toward or avoidance of natural worker mandibular gland extract delivered as an odor stream at the feeder.

Unlike natural aggression (Figure 3), pinching significantly increased hive visit duration (Figure 4). Recently, Lau and Nieh [24] found that signalers produce more stop signals when they spend longer inside the nest. This pattern may explain why pinched foragers increased signal production twice as much as victims of conspecific attack. In addition, degree of peril may be involved. Capture by a predator such as a crab spider generally results in death [33], whereas fights between conspecifics (Figure 2C) did not result in mortality in my trials. Thus, the cost of attempted predation may be higher than conspecific aggression, contributing to the larger signaling response for pinching. Finally, it would not be surprising if being attacked by a large predator (the human assistant) with unusually large "mandibles" (tweezers) contributed to forager reluctance to leave the nest, higher signaling levels, or both.

Natural Context

A negative feedback signal that can reduce recruitment to a dangerous site benefits the colony by preventing misallocation of resources and reducing individual mortality. A wide variety of predators such as praying mantids (Mantidae [28]), predacious bugs (Hemiptera [29]), bee-wolf wasps [37, 38], some social wasps [30], and, occasionally, bee-eating birds (Meropidae [39]) can capture bees at natural resources. Such attacks could explain why stop signalers targeted foragers visiting natural floral resources [22]. Attacks also occur when bees rob another colony's honey [25–27]. Interestingly, an artificial feeder that provides virtually unlimited, high-sugar-content food at a specific spatial point is more like a colony being robbed than a natural floral patch. Thus,

previous studies feeder studies may have simulated honey robbing.

In summary, a forager's experience at a patch [26] and her foraging motivation [40] influence her decision to recruit. For example, honey bees perform fewer waggle runs after returning from dangerous as compared to safe flowers [41]. However, one individual's decision to cease recruiting does not stop recruitment by other waggle dancers. By sending stop signals, she can inform foragers visiting the same location of adverse foraging conditions and provide negative feedback to counteract waggle dancing by others. Thus, collective actions of the superorganism arise from the positive and negative feedback of multiple actors, with negative feedback cycles providing greater precision and speed for labor reallocation. Indeed, the superorganism concept draws direct analogies between intercellular cooperation and teamwork between autonomous multicellular agents. It would not be surprising if negative feedback signals play an equally important role in self-organizing behaviors at the superoganism level, as they do within and between cells.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2009.12.060.

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References

1. Kholodenko, B.N. (2006). Cell-signalling dynamics in time and space. Nat. Rev. Mol. Cell Biol. *7*, 165–176.
2. Fewell, J.H. (2003). Social insect networks. Science *301*, 1867–1870.
3. Boomsma, J.J., and Franks, N.R. (2006). Social insects: From selfish genes to self organisation and beyond. Trends Ecol. Evol. *21*, 303–308.
4. Sumpter, D.J. (2006). The principles of collective animal behaviour. Philos. Trans. R. Soc. Lond. B Biol. Sci. *361*, 5–22.
5. Marshall, J.A.R., and Franks, N.R. (2009). Colony-level cognition. Curr. Biol. *19*, R395–R396.
6. Franks, N.R., Dornhaus, A., Best, C.S., and Jones, E.L. (2006). Decision making by small and large house-hunting ants colonies: One size fits all. Anim. Behav. *72*, 611–616.
7. Passino, K.M., Seeley, T.D., and Visscher, P.K. (2008). Swarm cognition in honey bees. Behav. Ecol. Sociobiol. *62*, 401–414.
8. Marshall, J.A.R., Bogacz, R., Dornhaus, A., Planqué, R., Kovacs, T., and Franks, N.R. (2009). On optimal decision-making in brains and social insect colonies. J. R. Soc. Interface *6*, 1065–1074.
9. Dornhaus, A., Brockmann, A., and Chittka, L. (2003). Bumble bees alert to food with pheromone from tergal gland. J. Comp. Physiol. [A] *189*, 47–51.
10. von Frisch, K. (1967). The Dance Language and Orientation of Bees, 2nd printing, 1993 Edition (Cambridge, MA: Belknap Press).
11. Sumpter, D.J.T., and Beekman, M. (2003). From nonlinearity to optimality: Pheromone trail foraging by ants. Anim. Behav. *66*, 273–280.
12. Robinson, E.J.H., Ratnieks, F.L.W., and Holcombe, M. (2008). An agent-based model to investigate the roles of attractive and repellent pheromones in ant decision making during foraging. J. Theor. Biol. *255*, 250–258.
13. Robinson, E.J.H., Jackson, D.E., Holcombe, M., and Ratnieks, F.L.W. (2005). Insect communication: 'No entry' signal in ant foraging. Nature *438*, 442.
14. Robinson, E.J.H., Green, K.E., Jenner, E.A., Holcombe, M., and Ratnieks, F.L.W. (2008). Decay rates of attractive and repellent pheromones in an ant foraging trail network. Insectes Soc. *55*, 246–251.
15. Sherman, G., and Visscher, P.K. (2002). Honeybee colonies achieve fitness through dancing. Nature *419*, 920–922.
16. Mattila, H.R., Burke, K.M., and Seeley, T.D. (2008). Genetic diversity within honeybee colonies increases signal production by waggle-dancing foragers. Proc Biol Sci *275*, 809–816.
17. Kirchner, W.H. (1993). Vibrational signals in the tremble dance of the honeybee, *Apis mellifera*. Behav. Ecol. Sociobiol. *33*, 169–172.
18. Michelsen, A., Kirchner, W.H., and Lindauer, M. (1986). Sound and vibrational signals in the dance language of the honeybee, *Apis mellifera*. Behav. Ecol. Sociobiol. *18*, 207–212.
19. Thom, C., Gilley, D.C., and Tautz, J. (2003). Worker piping in honey bees (*Apis mellifera*): The behavior of piping nectar foragers. Behav. Ecol. Sociobiol. *53*, 199–205.
20. Nieh, J.C. (1993). The stop signal of honey bees: Reconsidering its message. Behav. Ecol. Sociobiol. *33*, 51–56.
21. Esch, H.E. (1964). Beiträge zum Problem der Entfernungsweisung in den Schwänzeltanzen der Honigbiene. Z. Vgl. Physiol. *48*, 534–546.
22. Pastor, K.A., and Seeley, T.D. (2005). The brief piping signal of the honey bee: Begging call or stop signal? Ethology *111*, 775–784.
23. Seeley, T.D., and Tautz, J. (2001). Worker piping in honey bee swarms and its role in preparing for liftoff. J. Comp. Physiol. [A] *187*, 667–676.
24. Lau, C., and Nieh, J.C. (2010). Honey bee stop-signal production: Temporal distribution and effect of feeder crowding. Apidologie (Celle) *41*, 87–95.
25. Couvillon, M.J., Robinson, E.J.H., Atkinson, B., Child, L., Dent, K.R., and Ratnieks, F.L.W. (2008). En garde: Rapid shifts in honeybee, *Apis mellifera*, guarding behaviour are triggered by onslaught of conspecific intruders. Anim. Behav. *76*, 1653–1658.
26. Seeley, T.D. (1985). Honeybee Ecology (Princeton, NJ: Princeton University Press).
27. Winston, M.L. (1987). The Biology of the Honey Bee (Cambridge, MA: Harvard University Press).
28. Caron, D.M., and Ross, K.G. (1990). Spiders and pseudoscorpions. In Honey Bee Pests, Predators, and Diseases, R.A. Morse and R. Nowogrodzki, eds. (Ithaca, NY: Cornell University Press), pp. 177–187.
29. Greco, C.F., and Kevan, P.G. (1995). Patch choice in the anthophilous ambush predator *Phymata americana*: Improvement by switching hunting sites as part of the initial choice. Can. J. Zool. *73*, 1912–1917.
30. De Jong, D. (1990). Insects: Hymenoptera (ants, wasps, and bees). In Honey Bee Pests, Predators, and Diseases, R.A. Morse and R. Nowogrodzki, eds. (Ithaca, NY: Cornell University Press), pp. 135–155.
31. Dukas, R., and Morse, D.H. (2005). Crab spiders show mixed effects on flower-visiting bees and no effect on plant fitness components. Ecoscience *12*, 244–247.
32. Morse, D.H. (1981). Prey capture by the crab spider *Misumena vatia* (Clerck) (Thomisidae) on three common native flowers. Am. Midl. Nat. *105*, 359–367.
33. Morse, D.H. (1986). Predatory risk to insects foraging at flowers. Oikos *46*, 223–228.
34. Dukas, R. (2004). Effects of predation risk on pollinators and plants. In Cognitive Ecology of Pollination: Animal Behavior and Floral Evolution, L. Chittka and J.D. Thomson, eds. (Cambridge, UK: Cambridge University Press), pp. 214–236.
35. Seeley, T.D. (1992). The tremble dance of the honey bee: Message and meanings. Behav. Ecol. Sociobiol. *31*, 375–383.
36. Vallet, A., Cassier, P., and Lensky, Y. (1991). Ontogeny of the fine structure of the mandibular glands of the honeybee (*Apis mellifera* L.) workers and the pheromonal activity of 2-heptanone. J. Insect Physiol. *37*, 789–804.
37. Evans, E.E., and O'Neill, K.M. (1988). The Natural History and Behavior of North American Beewolves (Ithaca, NY: Cornell University Press).
38. Simonthomas, R.T., and Simonthomas, A.M.J. (1980). *Philanthus triangulum* and its recent eruption as a predator of honeybees in an Egyptian oasis. Bee World *61*, 97–107.
39. Fry, C.H. (1983). Honeybee predation by bee-eaters, with economic considerations. Bee World *64*, 65–78.
40. Barron, A.B., Schulz, D.J., and Robinson, G.E. (2002). Octopamine modulates responsiveness to foraging-related stimuli in honey bees (Apis mellifera). J. Comp. Physiol. [A] *188*, 603–610.
41. Abbott, K.R., and Dukas, R. (2009). Honeybees consider flower danger in their waggle dance. Anim. Behav. *78*, 633–635.

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Supplemental Information

A Negative Feedback Signal That Is Triggered by Peril Curbs Honey Bee Recruitment

James C. Nieh

Supplemental Experimental Procedures

Colonies and study sites

Experiments were conducted at UC San Diego (N32º53.127' and W117º13.785') from July to December 2007. On each day, one trial was conducted, beginning at 10:00. I sequentially used two honey bee colonies (*Apis mellifera* Linnaeus, 3000-4000 workers), inside a temperature-controlled room (30°C) with a 0.5 m long tube allowing the bees external access. The three-comb observation hive had a metal slide directing bees to the side on which they recruited (waggle danced). Assistants trained foragers to an inverted-jar feeder that could accommodate over 40 foragers [24]. They allowed 10 bees to visit the feeder, censused the feeder each 15 min and aspirated excess bees. The feeder provided 2.5 M unscented sucrose solution (65% sucrose w/w). Floral nectars occur at a variety of concentrations, and generalist bee foragers collect nectars ranging from 10-70% sugar w/w [S1].

Assistants uniquely marked all feeder bees with paints and determined if they came from the focal colony (residents) by watching for their return to the colony. A focal forager is a randomly chosen resident. Occasionally, bees from other colonies (competitors) would arrive at the feeder. There are no known apiaries within several miles of the experimental site, and thus competitors were presumably from feral colonies. If an unmarked bee fought with known focal-colony foragers, it was considered a competitor. Marked bees that did not return to the focal colony were also considered competitors. All competitors were immediately removed upon their subsequent return to the feeder, except during the competition phase of the competition experiment (see below).

During experiments, assistants exposed the dance-floor side of the colony to record sounds and videotape behavior (model PV-DV402D, Panasonic, Secaucus, New Jersey, USA). A hive visit began when a bee entered the comb region of the nest and ended when she left (through the entrance tube or room window). Assistants tracked the focal bee with a microphone (model: 33-3013, Radio Shack, Fort Worth, Texas, USA) held approximately 1 cm above her thorax. Digital video was analyzed with iMovie software (v5.0.2, Apple Computer, Cupertino, California, USA). Hive visit duration and wait time to first food unloading were recorded, in addition to the number of stop signals, waggle dance circuits, and percentage of hive visits with tremble dancing (tremble dancing scored as present or absent during a hive visit).

Stop signal specificity experiments

If stop signals convey information about a specific location, they should be directed towards foragers visiting the same site. I tested this with one colony by training bees from to two identical feeders (10 bees/feeder), both 100 m from the colony, and differing only in direction (north or south) and scent (lemon or peppermint, McCormick & Co., Hunt Valley, Maryland, USA). To elicit stop signal production, the assistant pinched the focal forager on her left metathoracic femur for 2 s while she was on the feeder (see below for rationale). All bees were individually marked and the same colors were used in different combinations at both sites to avoid paint-odor differences.

Bees can acquire the scent of food sources on their body hairs and nestmates can learn these acquired scents [10]. I therefore tested the hypothesis that signalers target bees that smell like the food location. Assistants directly applied a high dose of scent to ensure that each bee bore a strong, unambiguous odor (1 μl of scent to the dorsal abdomen upon landing: one application per visit, only one scent type for any given bee). Bees were not disturbed by scent application.

Two experiments were performed: *different odor* at each feeder (78 focal bees) and *same odor* at both feeders (77 focal bees). In the different-odor experiment, each location had a different odor. In

the same-odor experiment, all combinations of odor and location were used. Odor was randomly assigned to a particular location at the beginning of each trial. Assistants only changed odors between trials (between different days). The hive visit of each focal bee was recorded only once, and new bees were trained without odor application at the end of each trial. For both experiments, a nest observer tracked the focal forager with a video camera and microphone, recording all stop signals that she produced and receiver identity.

During these trials, there was natural food dearth, and all flight activity and recruitment dances were for the feeders. Stop signalers remained on the dance floor when inside the nest. Non-foragers (called "other bees") also received stop signals. During trials, all foragers experienced with the feeder were marked and were either actively foraging or captured in aspirators. Thus, the "other bees" were not feeder foragers from previous days. The population of bees on the two combs comprising the dance floor was estimated to be 1100 (based on four censuses within a 10 cm^2 square placed at random locations).

Competition experiment

In the competition phase, focal foragers are defined as victims (they were attacked by competitors), aggressors (they attacked competitors), or undisturbed (no aggression received or given). Assistants trained bees 100 m north of the focal colony, randomly selected a focal bee that fed in the absence of competitors, and recorded her subsequent hive visit (before competition phase). The no-competition period ended when competitors appeared and lasted approximately 60 min. Focal forager behavior was then recorded after competition had begun (after phase). Assistants thus obtained before and after nest visits from 20 foragers (10 per colony) in each category: victims, aggressors, and undisturbed.

Assistants allowed competitors to feed and increase to a maximum of approximately 20 bees. I used small colonies (3000-4000 workers) that could not defend the feeder against typically larger feral

honey bee colonies (16000±3510 workers, [S2]). Thus, controlled removal prevented competitors from overwhelming the feeder. With only 20 competitors, focal colony foragers continued to feed because feeder capacity was more than 40 bees.

At the end of each trial, assistants removed all competing bees, waiting 1 hr to ensure removal of most competitors. On the next trial day, a small number of competitors that were not removed on the previous day or scouts from competing colonies generally found the feeder. This experiment was conducted at the end of the study period when natural resources were scarcer and the rate of feral colonies discovering the feeder was highest. In all other experiments, less than 5% of feeder bees were feral.

Physical aggression experiment

Biting plays an important role in aggression during food competition (this study) and attempted predation [34]. Assistants pinched bees to simulate the effect of biting, used a different pair of clean forceps for each bee in each phase. To control for tweezer exposure, a pair of clean tweezers was held 2 mm next to, but not touching, the left metathoracic leg of a focal bee for 2 s in the before phase. Upon her next feeder visit, fine forceps were used to pinch her left metathoracic femur for 2 s, applying sufficient force to prevent escape, but not damaging the leg. To determine if this pinching wounded the leg, we observed movement of the left metathoracic leg as the bee walked around the nest and saw no change in how this leg was used or moved before and after pinching. We also recorded the nest behavior of pinched bees after four subsequent and consecutive trips to the feeder in case pinching wounded the bees and prevented waggle dancing. Waggle dancing increased consistently with each successive feeder visit, increasing on average by 54 fold after the fourth feeder trip (average elapsed time of 23 min after pinching).

Gland extract experiments

During attacks, bees released alarm pheromone from their sting glands [27] that human assistants and other bees detected. To test the effect of odors alone, assistants used sting and mandibular gland extracts. The function of worker mandibular glands is unclear, but it does not elicit alarm behavior, and is not involved in aggression [36]. Mandibular gland extract therefore served as a control for the aggression-related signal provided by alarm pheromone.

Extracts were prepared by dissecting out the mandibular glands (two per bee) and sting gland (one per bee) from a cold-anesthetized nestmate captured as she left the nest. Dissected glands were crushed with hexane (100 μl per sting gland and 50 μl per mandibular gland, H302-1, Fisher Scientific, Pittsburgh, Pennsylvania, USA) in tissue homogenizers) and stored in sealed glass vials at 0°C for 12 hrs. To avoid cross-contamination, separate dissections were conducted with different extract and applicator equipment for mandibular and sting glands.

Before each trial, assistants prepared separate sets of control and extract odor applicators, each consisting of a 10 ml syringe with filter paper onto which they dispensed 100 μl of hexane (control) or 100 μl of extract (one-bee equivalent of sting or mandibular extract). Applicators were kept on ice in the field. In the before phase, assistants applied a control hexane-only odor stream for 1 min (40 ml/min, four repeated plunges of the same syringe) placed 1 cm above a focal forager's antennae. Upon her subsequent feeder visit, the same forager received the gland extract (after phase). If she flew away, subsequent plunges were applied when she returned (total exposure time of 1 min). Control and extract syringes were used for only one application period.

Statistical methods

I analyzed data with JMP (v7.0.1, SAS software, Cary, North Carolina, USA) and report averages as mean (±1 standard deviation). Signal specificity and overall stop-signal production data (competition experiment) met parametric assumptions. For the specificity experiments, I used analysis of variance

(ANOVA) with receiver type and sender location as fixed effects. In the same-odor experiment, different odors were applied to the same location and I tested the effect of odor type (fixed effect). Chi-square tests were used to determine if signals are targeted at specific receiver types. For all other data, I performed repeated-measures analyses with Wilcoxon signed ranks tests, reporting 2-tailed *P*-values and applied a Sequential Bonferroni correction (two tests performed on each data set, tests that fail the correction reported as "NS_{SB}"). In the competition, physical aggression, and gland extract experiment, there were no significant colony-based differences in forager behavior ($W_{20} \leq 50.0$, $P \geq 0.06$). Results from both colonies were therefore pooled in subsequent analyses.

Supplemental References

S1. Roubik, D.W., Yanega, D., Aluja, S.M., Buchmann, S.L., and Inouye, D.W. (1995). On optimal nectar foraging by some tropical bees (Hymenoptera: Apidae). Apidologie *26*, 197-211.

S2. Seeley, T.D., and Morse, R.A. (1976). The nest of the honey bee (*Apis mellifera* L.). Insectes Soc. *23*, 495-512.

minus-end directed kinesin, Ncd, which crosslinks and slides interpolar microtubules bundles; this activity pulls centrosomes back together, and thus acts as a brake for migration (Figure 1) [3,12]. Interestingly, inhibition of cytoplasmic dynein eliminates the initial fast phase of centrosome migration, but centrosomes are still capable of separating to roughly half their final interpolar distance [3]. Therefore, although a microtubule-motor component actively drives centrosome migration, these results argue for the existence of a second force-generating mechanism that shares the centrosome-separation duties with microtubules.

Cao and colleagues [9] show that dynamic actin turnover in the expanding cortical actin caps is an additional mechanism that drives interphase–prophase centrosome migration in these cells. Drug-induced F-actin depolymerization or stabilization results in a failure in both actin cap expansion and defects in centrosome migration. Likewise, disruption of either actin branching (by mutation of *Arpc1*, an Arp2/3 component [15]) or formin-mediated actin assembly (directly by mutation of *diaphanous* [16] or indirectly by injection of the RhoA inhibitor C3 exotransferase) significantly reduces actin cap expansion as well as the extent of centrosome migration. Strikingly, these authors also demonstrate that non-muscle myosin-II is not required for interphase–prophase centrosome migration. This was performed by microinjection of the Rho kinase inhibitor Y-27632. Thus, cortical (cap) expansion in this system is required for centrosome migration but, unlike the finding by Rosenblatt *et al.* [8], does not require myosin-II activity. Instead, actin dynamics appear to drive cortical cap expansion and the migration of the centrosomes to which they are attached. Furthermore, the authors demonstrate that cap expansion is not needed for further centrosome separation after NEBD [9], unlike in cultured mammalian cells in which myosin-II activity is utilized [8].

Notably, as with dynein/dynactin inhibition, suppression of F-actin dynamics did not entirely block centrosome migration [3,9]. In fact, embryos treated with latrunculin to depolymerize their cortical actin network could still partially separate their centrosomes (a 50% reduction relative to control). This begs the question: what is the relationship between cortical dynein and cap expansion in driving centrosome migration? Cortical dynein does co-localize with actin in the caps throughout cap expansion, but it is not known whether actin disruption displaces cortical dynein. Since centrosomes still partially migrate after embryos are microinjected with latrunculin, one possibility is that dynein localizes to the cortex in an actin-independent manner and is responsible for this limited movement (Figure 1). But if latrunculin disrupts both cortical dynein and F-actin, then what additional unknown mechanism is responsible for the observed centrosome migration? Future studies that focus on co-disruption of dynein activity and actin polymerization will be needed to resolve this important issue.

References

1. Walczak, C.E., and Heald, R. (2008). Mechanisms of mitotic spindle assembly and function. Int. Rev. Cytol. *265*, 111–158.
2. Wadsworth, P., and Khodjakov, A. (2004). E pluribus unum: towards a universal mechanism for spindle assembly. Trends Cell Biol. *14*, 413–419.
3. Sharp, D.J., Brown, H.M., Kwon, M., Rogers, G.C., Holland, G., and Scholey, J.M. (2000). Functional coordination of three mitotic motors in *Drosophila* embryos. Mol. Biol. Cell *11*, 241–253.
4. Sharp, D.J., Rogers, G.C., and Scholey, J.M. (2000). Microtubule motors in mitosis. Nature *407*, 41–17.
5. Sawin, K.E., and Mitchison, T.J. (1991). Mitotic spindle assembly by two different pathways *in vitro*. J. Cell Biol. *112*, 925–940.
6. Toyoshima, F., and Nishida, E. (2007). Spindle orientation in animal cell mitosis: roles of integrin in the control of spindle axis. J. Cell Physiol. *213*, 407–411.
7. Siller, K.H., and Doe, C.Q. (2009). Spindle orientation during asymmetric cell division. Nat. Cell Biol. *11*, 365–374.
8. Rosenblatt, J., Cramer, L.P., Baum, B., and McGee, K.M. (2004). Myosin II-dependent cortical movement is required for centrosome separation and positioning during mitotic spindle assembly. Cell *117*, 361–372.
9. Cao, J., Crest, J., Fasulo, B., and Sullivan, W. (2010). Cortical actin dynamics drive the early stage of centrosome separation. Curr. Biol. *20*, 770–776.
10. Foe, V.E., and Alberts, B.M. (1983). Studies of nuclear and cytoplasmic behavior during the five mitotic cycles that precede gastrulation in *Drosophila* embryogenesis. J. Cell Sci. *61*, 31–70.
11. Stevenson, V.A., Kramer, J., Kuhn, J., and Theurkauf, W.E. (2001). Centrosomes and the Scrambled protein coordinate microtubule-independent actin reorganization. Nat. Cell Biol. *3*, 68–75.
12. Cytrynbaum, E.N., Sommi, P., Brust-Mascher, I., Scholey, J.M., and Mogilner, A. (2005). Early spindle assembly in *Drosophila* embryos: role of a force balance involving cytoskeletal dynamics and nuclear mechanics. Mol. Biol. Cell *16*, 4967–4981.
13. Robinson, J.T., Wojcik, E.J., Sanders, M.A., McGrail, M., and Hays, T.S. (1999). Cytoplasmic dynein is required for the nuclear attachment and migration of centrosomes during mitosis in *Drosophila*. J. Cell Biol. *146*, 597–608.
14. Sharp, D.J., Rogers, G.C., and Scholey, J.M. (2000). Cytoplasmic dynein is required for poleward chromosome movement during mitosis in *Drosophila* embryos. Nat. Cell Biol. *2*, 922–930.
15. Stevenson, V., Hudson, A., Cooley, L., and Theurkauf, W.E. (2002). Arp2/3-dependent pseudocleavage [correction of psuedocleavage] furrow assembly in syncytial *Drosophila* embryos. Curr. Biol. *12*, 705–711.
16. Afshar, K., Stuart, B., and Wasserman, S.A. (2000). Functional analysis of the *Drosophila* diaphanous FH protein in early embryonic development. Development *127*, 1887–1897.

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Honeybee Communication: A Signal for Danger

Scout honeybees recruit other bees to visit a newly discovered food source through the famous 'waggle dance'. Now a new study reports that other nest mates can induce the dancer to stop advertising, if they have experienced danger at that location.

Mandyam V. Srinivasan

Over the years, the 'waggle dance' of the honeybee has come to be regarded as a textbook example of the ability of relatively small and simple organisms to communicate with each other in a surprisingly abstract and symbolic fashion [1]. When a honeybee has discovered a new, attractive source of nectar or pollen, she returns to the hive and performs this dance to advertise this discovery to her nest mates, and to convey to them the exact position of

the food source, so that they may also forage from it. The dance encodes, in symbolic fashion, how far and in which direction her potential recruits should fly to find the food source. Now, a new study by James Nieh [2], published in a recent issue of *Current Biology*, has revealed that other nest mates, watching this dance, are able to make the dancer discontinue her advertisement of the food source if they had experienced danger or conflict when they visited it.

The Waggle Dance

In her waggle dance, a honeybee conveys the position of the food source from which she has just returned, in terms of its distance and direction relative to the nest. In the dance, which is performed on the vertical surface of the honeycomb, the bee moves in a series of alternating left- and right-hand loops, roughly tracing a figure of eight (Figure 1). At the end of each loop, the bee enters a so-called 'waggle' phase in which she waves her abdomen rapidly from side to side. The angle between the axis of the waggle and the vertical direction represents the angle between the sun and the direction in which a bee should fly in order to find the goal. The duration of the waggle phase is proportional to the distance of the food source from the hive.

The dancing bee conveys information about the location of the food source to her nest mates in this highly symbolic way, with the vertically upward direction representing the direction of the sun [1]. Other bees, following closely behind the dancer, are able to glean this navigational information, and some of them are sufficiently persuaded by the advertisement to seek out the food source for themselves. If the new recruits find the food source and are sufficiently 'enthusiastic' about their bounty, they, too, perform the waggle dance upon returning to the hive, to persuade a further group of bees to visit the food source. Consequently, the number of visitors to the food increases exponentially with time. On the other hand, when a source of nectar has dried up or is past its prime, bees returning from it no longer dance, and eventually stop visiting it. Thus, the recruitment and the visits to a food source are shut off rapidly when its profitability declines. In this way, the colony is able to quickly direct its foraging resources to new or better targets, as they emerge.

Figure 1. The stopping of a waggle dance.
The waggle dancer (with yellow and pink paint marks) is frozen at the moment of receiving a stop signal from the bee denoted by 'S' to her left. There is physical contact between the head of the stop signaler and the body of receiving waggle dancer. Image courtesy of James C. Nieh.

A 'Danger' Signal

The new study by Nieh [2] reveals that a bee that has had a traumatic or 'unpleasant' experience at a food site — such as an injury, or an attack from another insect or bee — can generate a warning signal to prevent other bees from being recruited to visit that site. She does this by butting her head against a dancing bee that is advertising the site, and emitting a brief buzzing tone [3]. This 'danger' signal causes the dancer to stop dancing, and hence to stop further recruitment to that site.

What constitutes such an 'unpleasant' experience for a bee when it feeds at a flower? An attack by a waiting spider, a mantid, or a predacious bug would be one kind of example. Such predators often keep station at nectar-bearing flowers to ambush visiting bees. Another example of an undesirable experience would be a debilitating fight with a bee visiting the flower from another colony. These fights arise because colonies often compete for the same food source, and bees distinguish between their own hive mates and other bees by sensing their body (cuticular) odours: bees from different colonies carry different olfactory signatures. Nieh [2] finds that gently pinching the leg of a bee (to simulate a bite from another insect) while it visits a feeder can induce the bee to direct 'danger' signals toward dancing bees when it returns to the hive [2]. In the case of fights between rival-colony bees, Nieh finds, interestingly, that a bee returning from a fight will signal danger only when she has lost a battle and is wounded, not when she has won and returned uninjured [2]. Another stimulus that evokes the perception of danger in a visiting bee is exposure to the so-called 'alarm pheromone' — a pheromone that bees exude when they perceive threat or are in a behaviourally aggressive state. A puff of this pheromone, delivered to a bee when she visits the feeder, causes her to stop other bees from dancing to advertise the food source when she returns home [2].

Are there any particular dancers toward which the traumatized bee directs her warning signals? In a beautifully designed experiment, Nieh [2] trained two groups of individually marked bees, from the same colony, to visit differently scented feeders. One group was trained to feeder A, which carried the scent of lemon. Another group was

trained to feeder B, which had the scent of peppermint. The feeders were positioned at different locations with respect to the hive. Pinching the leg of a bee visiting feeder A caused the bee to preferentially direct its danger signals toward dancing bees that were advertising the location of feeder A and carried the scent associated with A. Similarly, bees pinched while visiting feeder B targeted their danger signals toward bees advertising feeder B, based on the scent that they carried [2]. That it was indeed the scents that provided the crucial piece of information is evident from the fact that this targeting specificity disappeared when the experiment was repeated using identical scents at both feeders. A pinched bee returning from either feeder location would then direct a danger signal at any dancing bee that carried the scent, which was now common to both feeders [2]. Thus, the targeting of the 'danger' signal was driven by the *scent* on the dancer's body, and not by the *location* that she was indicating in her dance. Interestingly, pinched bees also occasionally delivered danger signals to non-dancing foragers, or even to bees that had not visited either site [2]. This may be because the targeting was not 100% accurate (as suggested by the author) or because the danger signal could be a more broadly directed message to all bees in the colony, saying "*Don't visit any food source that smells like me!*" It is now well established that scent alone can trigger recall of specific feeding locations in honeybees [4–6].

It has been known for some time that honeybees produce signals to cause bees to stop dancing bees in various other contexts. For example, bees returning from an excessively crowded feeder often produce an acoustic signal that is similar to the 'danger' signal described above. This causes waggle-dancing bees to freeze momentarily, and then to discontinue their dance [7–10]. Presumably, this serves to prevent or reduce the recruitment of even more bees to a food source that is becoming difficult and time-consuming to access. In another context, bees returning in large numbers from a plentiful supply of food perform a so-called 'tremble' dance, which is thought to be a call to urge more of the hive's nectar-uptake bees to contribute the task of offloading the nectar from the foragers when it is arriving at a very high rate [1,11]. This signal ensures that, at the colony level, the rate of uptake of the nectar within the hive matches the rate at which it is flowing into the hive. However, it has recently been noticed that tremble dancers also emit buzzes similar to the 'danger' signals described above, and that these signals again cause cessation of waggle-dancing in the hive [7,8]. These signals may serve to stem the recruitment of foragers to a food site from which nectar is already coming in at an unmanageably high rate. More generally, it appears that the 'stop' signal acts to discourage visits to a food source that is no longer profitable for the colony to exploit, for a variety of reasons. Finally, recent work is suggesting that the so-called 'begging' signals, which were believed to be used by a dance-follower to request a taste of the nectar that a dancer had just brought in [1,12], may not be a begging signal after all, but just a 'stop' signal. The reason for this new interpretation is that, although the dancer stops dancing in response to the so-called 'begging' signals, she rarely obliges the 'beggar' with a nectar sample [7,13].

Communication in honeybees turns out to be vastly more sophisticated than originally imagined. Research is revealing a variety of subtle, interwoven feedback loops that act, through the behaviour of individual bees, to provide the colony with a collective intelligence that endows it with a capacity to adapt quickly and appropriately to changes in the foraging environment [14]. The 'danger' signal uncovered by Nieh's study [2] adds another word to the rich and growing vocabulary of honeybee communication. Indeed, it makes one pause to ask whether these creatures may be more than just simple, reflexive, unthinking automata.

References

1. von Frisch, K. (1993). The Dance Language and Orientation of Bees (Cambridge, MA: Harvard University Press).
2. Nieh, J.C. (2010). A negative feedback signal that is triggered by peril curbs honeybee recruitment. Curr. Biol. *20*, 310–315.
3. Michelsen, A., Kirchner, W., and Lindauer, M. (1986). Sound and vibration signals in the dance language of the honeybee, *Apis mellifera*. Behav. Ecol. Sociobiol. *18*, 207–212.
4. Reinhard, J., Srinivasan, M.V., and Zhang, S.W. (2004). Scent-triggered navigation in honeybees. Nature *427*, 411.
5. Reinhard, J., Srinivasan, M.V., Guez, D., and Zhang, S.W. (2004). Floral scents induce recall of navigational and visual memories in honeybees. J. Exp. Biol. *207*, 4371–4381.
6. Reinhard, J., Srinivasan, M.V., and Zhang, S.W. (2006). Complex memories in honeybees: Can there be more than two? J. Comp. Physiol. A *192*, 409–416.
7. Nieh, J.C. (1993). The stop signal of honeybees: Reconsidering its message. Behav. Ecol. Sociobiol. *33*, 51–56.
8. Kirchner, W.H. (1993). Vibrational signals in the tremble dance of the honeybee, *Apis mellifera*. Behav. Ecol. Sociobiol. *33*, 169–172.
9. Thom, C., Gilley, D.C., and Tautz, J. (2003). Worker piping in honeybees (*Apis mellifera*): The behavior of piping nectar foragers. Behav. Ecol. Sociobiol. *53*, 199–205.
10. Lau, C., and Nieh, J.C. (2010). Honeybee stop-signal production: Temporal distribution and effect of feeder crowding. Apidologie *41*, 87–95.
11. Seeley, T.D. (1992). The tremble dance of the honeybee: message and meanings. Behav. Ecol. Sociobiol. *31*, 375–383.
12. Esch, H.E. (1964). Beiträge zum Problem der Entfernungsweisung in den Schwänzeltänzen der Honigbiene. Z. vergl. Physiol. *48*, 534–546.
13. Pastor, K.A., and Seeley, T.D. (2005). The brief piping signal of the honeybee: Begging call or stop signal? Ethology *111*, 775–784.
14. Seeley, T.D. (1995). The Wisdom of the Hive: The Social Physiology of Honeybee Colonies (Cambridge, MA: Harvard University Press).

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Ribosomal Genes: Safety in Numbers

The presence of inactive units in tandem arrays of ribosomal genes (rDNA) has been linked to increased transcriptional capacity, but a recent study indicates that inactive units are necessary for sister chromatid cohesion and genetic stability of rDNA.

Luis Aragón

Protein synthesis requires several million of new ribosomes per generation, hence cells need to synthesize vast amounts of ribosomal (r)RNAs. When cells need to progress rapidly through the cell cycle — as for example in early development — or when they find themselves under stress