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SOCIAL LEARNING

Social signal learning of the waggle dance in honey bees

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Honey bees use a complex form of spatial referential communication. Their “waggle dance” communicates the direction, distance, and quality of a resource to nestmates by encoding celestial cues, retinal optic flow, and relative food value into motion and sound within the nest. We show that correct waggle dancing requires social learning. Bees without the opportunity to follow any dances before they first danced produced significantly more disordered dances with larger waggle angle divergence errors and encoded distance incorrectly. The former deficit improved with experience, but distance encoding was set for life. The first dances of bees that could follow other dancers showed neither impairment. Social learning, therefore, shapes honey bee signaling, as it does communication in human infants, birds, and multiple other vertebrate species.

Social learning occurs when one individual learns by observing or interacting with another (1) and is particularly useful when complex behaviors must be tuned to specific environmental circumstances or honed by practice or social shaping. For example, human infant babbling and young songbird subsongs are shaped by social feedback into more mature vocal behavior (2), and young naked mole rats learn distinctive colony dialects from older rats (3). Longer periods of interaction, such as those occurring between parents and offspring, can favor the evolution of such open programs (4), which allow novices to acquire skills more rapidly from experienced individuals than they could on their own (5). Proficient individuals have had more opportunities to fine-tune their brains and motor outputs to environmental circumstances (5); thus, learning from them can be beneficial.

Eusocial insects use social learning, but it is unclear whether this learning shapes their communication, which can be remarkably sophisticated and cognitively complex. *Polistes fuscatus* wasps use social eavesdropping, a form of social learning, to observe conflicts and to assess and remember rivals through facial recognition (6). Bumble bees can learn by observation to copy or avoid the foraging choices of other bumble bees through their previous experiences of reward or punishment (7). These bees can also learn to obtain a nectar reward by watching their nestmates perform a new behavior and can then innovate and solve the problem more efficiently (8). Honey bee workers use social learning when following the waggle dance to

learn resource location and quality. However, it has not been previously determined whether dance following can improve the dance performances of young waggle dancers or whether the dance is completely genetically preprogrammed (innate).

The waggle dance is a sophisticated form of spatial referential communication (9). The dancer repeatedly circles in a figure-eight pattern centered around a waggle run in which the bee waggles its abdomen as it moves forward (Fig. 1). Referential communication codes information, and the dancer encodes the po-

lar coordinates of a resource relative to the nest. Longer waggle runs communicate greater distances (more retinal optical flow), and the waggle direction angle communicates resource direction. When a bee dances on a vertical comb in the dark, the bee points in the direction of the resource relative to the sun, as transposed to the vertical in relation to gravity. The quality of the food relative to colony need and the dancer’s prior experiences (10) are encoded in the number of waggle run repetitions and the speed with which the dancer returns to repeat each successive waggle run (11).

There is a strong genetic component to the dance: Different honey bee species have distinctive distance encodings (calibrations) that persist even when they are cross-fostered (12, 13). An encoding is a curve that describes the relationship between physical distance and the duration of waggle runs for resources at those distances (14). Theoretically, novice dancers could benefit by learning from experienced dancers because waggle dancing requires retrieving navigational memory and using detailed motor programs and real-time feedback to translate resource location (15). Dances occur on the dance floor, which often consists of colony-specific, uneven, and convoluted comb surfaces (Fig. 1 and fig. S1) (16) that dancers must negotiate at relatively high velocities. On average, they cover more than their body length in 1 s (waggle running at

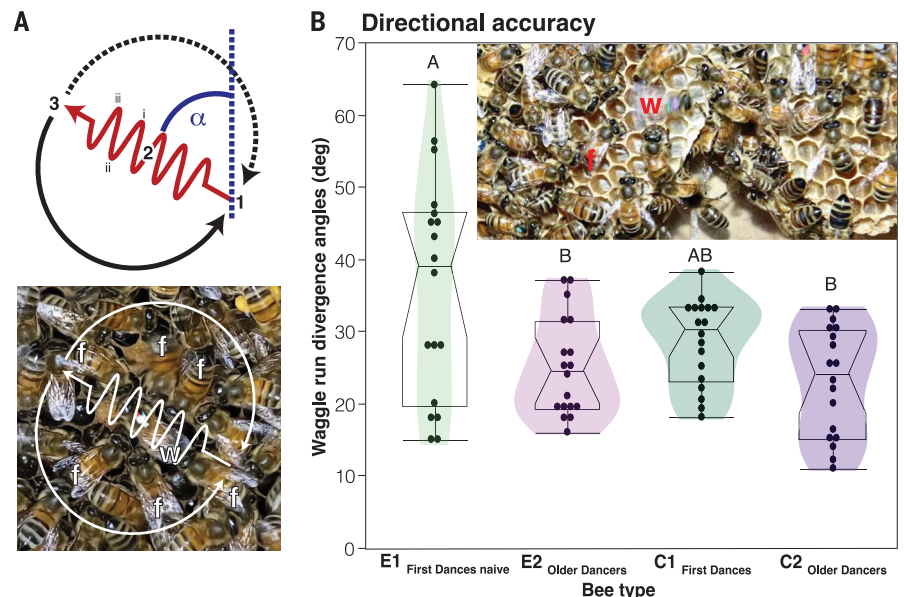


Fig. 1. Waggle dance directional error was highest in the first dances of naive bees that could not follow waggle dances. (A) The dancer (w) shakes its abdomen (i-ii-iii, creating one cycle) during the waggle run (1-2-3), whose angle (α) communicates direction, and then makes a semicircular return while being tracked by dance followers (f). (B) Divergence error angles decreased with experience in experimental colonies but not in control colonies, in which errors were consistently low (different letters indicate significant differences, Tukey HSD test, $P < 0.05$). (Inset) Dancers typically perform on irregular surfaces that vary between colonies. Data (black circles), notched box plots, and violin plots are shown in all figures.

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15 mm/s and returning back at 20 mm/s) while sensing the positions of their bodies relative to gravity and producing the correct waggle frequency and angle (17). Thus, errors occur. A dancer's successive waggle runs can point to different angles, resulting in directional errors (18). Similarly, waggle runs within the same dance can vary in duration, conveying distance errors (19).

Foragers have the opportunity to learn from more experienced dancers. Workers become foragers as they age: They begin following waggle dancers when they are 8 days old and subsequently perform their first waggle dances when they are 12 days old (20). All workers follow waggle dances before they waggle dance (20), and most follow dances performed by older bees that have previously danced (11). We therefore predicted that the first waggle dances of foragers will exhibit more errors if they are reared in an environment in which they cannot follow other waggle dancers before they begin to dance.

We therefore created colonies in which we observed the first waggle dances produced by foragers (all individually marked and trained to 55% w/v sucrose feeders located 150 m from their colonies) that either could or could not follow other waggle dancers (table S1). Each of our five experimental colonies was established with a single cohort of 1-day-old bees. As these bees aged, we monitored the colonies until we observed the first waggle dances and then observed the same dancers 20 days later when they had more foraging and dancing experience. Naive dancers could not follow any other dancers before their first dances because all bees in the colony were the same age, but as these dancers grew older, they followed other waggle dancers and had more experience dancing. In five control colonies that we established at the same time with adult bees of all ages and in which we observed waggle dancing within 1 to 2 days of colony creation, we measured the waggle dances of control bees at two comparable stages: the first waggle dances in the control colonies ($C1_{\text{First Dances}}$) and the waggle dances of the same dancers 20 days later when they had more foraging and dancing experience ($C2_{\text{Older Dancers}}$).

We observed no waggle dancing in all experimental colonies before the first group of bees aged into foraging and dancing ($E1_{\text{First Dances naive}}$; 9.0 ± 2.0 days old). Although we did not track all behaviors of these same bees until 20 days later, when they were older and had experience dancing and following other dancers ($E2_{\text{Older Dancers}}$), on each observation day we saw multiple $E1_{\text{First Dances naive}}$ bees following waggle dancers for natural food sources. In all control colonies, we had a marked cohort of bees of known age and likewise observed that they followed waggle dances before they performed their first dances ($C1_{\text{First Dances}}$;

Table 1. Summary of statistical results for all experiments. Colony type is either experimental (E) or control (C), and time point refers to (1) the first dances of bees or (2) subsequent dances of the same bees observed 20 days later.

Measure	Model R^2_{adj}	Colony type	Time point	Interaction, colony type by time point
<i>Food direction</i>				
Divergence angle	0.12	$F_{1,30} = 5.85$, $P = 0.02$	$F_{1,34} = 12.93$, $P = 0.001$	$F_{1,34} = 1.52$, $P = 0.23$
<i>Food distance</i>				
Waggle duration	0.79	$F_{1,32} = 157.20$, $P < 0.0001$	$F_{1,34} = 0.67$, $P = 0.42$	$F_{1,34} = 1.32$, $P = 0.26$
Waggle duration range error	0.34	$F_{1,30} = 20.08$, $P = 0.0001$	$F_{1,34} = 14.99$, $P = 0.0005$	$F_{1,34} = 0.09$, $P = 0.77$
Waggle duration CV	0.02	$F_{1,30} = 0.03$, $P = 0.86$	$F_{1,34} = 10.18$, $P = 0.003$	$F_{1,34} = 0.28$, $P = 0.60$
Number of waggles per waggle run	0.57	$F_{1,27} = 88.26$, $P < 0.0001$	$F_{1,34} = 1.46$, $P = 0.24$	$F_{1,34} = 1.01$, $P = 0.32$
Number of waggles per waggle run CV	<0.001	$F_{1,28} = 0.87$, $P = 0.36$	$F_{1,34} = 3.83$, $P = 0.06$	$F_{1,34} = 0.94$, $P = 0.34$
Return flight time	0.52	$F_{1,29} = 8.56$, $P = 0.007$	$F_{1,34} = 22.80$, $P < 0.0001$	$F_{1,34} = 12.48$, $P = 0.0012$
<i>Food quality</i>				
Number of waggle runs	0.56	$F_{1,30} = 4.99$, $P = 0.03$	$F_{1,34} = 21.58$, $P < 0.0001$	$F_{1,34} = 1.35$, $P = 0.25$
Return-phase duration	0.12	$F_{1,30} = 11.68$, $P = 0.002$	$F_{1,34} = 15.15$, $P = 0.0004$	$F_{1,34} = 2.54$, $P = 0.12$
Return-phase CV	0.03	$F_{1,31} = 0.13$, $P = 0.72$	$F_{1,34} = 0.60$, $P = 0.45$	$F_{1,34} = 0.05$, $P = 0.82$
<i>Dance quality</i>				
Disorder proportion	0.08	$F_{1,30} = 7.35$, $P = 0.011$	$F_{1,34} = 20.43$, $P < 0.0001$	$F_{1,34} = 6.46$, $P = 0.02$
Number of followers	0.76	$F_{1,31} = 180.07$, $P < 0.0001$	$F_{1,34} = 49.60$, $P < 0.0001$	$F_{1,34} = 17.46$, $P = 0.0002$

9.9 ± 1.0 days old) and continued to follow waggle dances over the next 20 days. All statistical results are reported in Table 1.

Food direction and distance

$E1_{\text{First Dances naive}}$ bees had significantly greater divergence angles (higher directional error) that decreased when they became $E2_{\text{Older Dancers}}$ bees [Tukey honestly significant difference (HSD) test, $P < 0.05$, Fig. 1B]. The dances of $C1_{\text{First Dances}}$ and $C2_{\text{Older Dancers}}$ bees did not have significantly different divergence errors.

The dances of $E1_{\text{First Dances naive}}$ and $E2_{\text{Older Dancers}}$ bees had longer waggle run durations than those of $C1_{\text{First Dances}}$ or $C2_{\text{Older Dancers}}$ bees (Tukey HSD test, $P < 0.05$, Fig. 2), suggesting that distance encoding was disrupted when bees could not follow experienced dancers and that disruption persisted even after they had more practice dancing and following other dancers. The reasons for this disruption are unclear, but $E1_{\text{First Dances naive}}$ foragers had longer return flight times than those of all other bee types (Tukey HSD test, $P < 0.05$). If $E1_{\text{First Dances naive}}$ bees thereby experienced

greater retinal optic flow, this should translate into longer waggle run durations (21). However, when the same bees were 20 days older, they had shorter flight durations and yet persisted in making the same distance-encoding errors.

The waggle duration range error was significantly higher in the dances of $E1_{\text{First Dances naive}}$ bees than in those of $C1_{\text{First Dances}}$ or $C2_{\text{Older Dancers}}$ bees (Tukey HSD test, $P < 0.05$), although it was not different between $E1_{\text{First Dances naive}}$ and $E2_{\text{Older Dancers}}$ bees, again suggesting a lifetime disruption of distance communication as a result of our treatment. In accordance with the waggle duration trends, the dances of $E1_{\text{First Dances naive}}$ and $E2_{\text{Older Dancers}}$ bees had more waggles per waggle run than those of $C1_{\text{First Dances}}$ or $C2_{\text{Older Dancers}}$ bees (Tukey HSD test, $P < 0.05$). There were no significant differences between coefficients of variation (CV) for waggle run duration or the number of waggles per waggle run (Tukey HSD test, $P > 0.05$).

Food quality

Bees signal higher-quality food relative to colony needs by increasing the number of waggle runs

per dance and performing shorter return phases (11). In general, our dancers tended to signal a higher value for identical sucrose solutions when their colonies were older and larger (E2_{Older Dancers} and C2_{Older Dancers} phases) than when their colonies were smaller and younger (Fig. 3A, E1_{First Dances naive} and C1_{First Dances} phases), perhaps reflecting greater colony need. The dances of E2_{Older Dancers} bees had significantly more waggle runs than those of E1_{First Dances naive} bees, but in control colonies there were no significant differences between the first waggle dances of bees and their waggle dances 20 days later (Tukey HSD tests, $P > 0.05$). Return-phase durations were only shorter for C2_{Older Dancers} bees as compared with all other forager types (Tukey HSD tests, $P < 0.05$). There were no significant differences in return-run duration CV (Tukey HSD test, $P > 0.05$).

Dance quality

The dances of E1_{First Dances naive} bees were significantly more disordered than the dances of E2_{Older Dancers}, C1_{First Dances}, or C2_{Older Dancers} bees (Tukey HSD test, $P < 0.05$, Fig. 3B). The number of dance followers per dance was significantly lower for experimental colonies than for control colonies (Tukey HSD test, $P < 0.05$), but was not different between E1_{First Dances naive} and E2_{Older Dancers} bees. Increasing dance disorder was positively correlated with higher divergence angle errors for E1_{First Dances naive} and C2_{Older Dancers} bees ($F_{1,16} \geq 4.72$, $P \leq 0.045$) but not for E2_{First Dances} or C1_{First Dances} bees ($F_{1,16} \leq 0.43$, $P \geq 0.52$, Fig. 3C).

Our results suggest that social signal learning can improve waggle dancing. The dances of E1_{First Dances naive} bees who could not follow dances before they first danced had greater divergence angle errors, signaled greater distances, and were significantly more disordered than those of C1_{First Dances} bees that were exposed to waggle dancing. Once the same bees were older and had experience with dance following and dancing (E2_{Older Dancers}), they significantly decreased divergence angle errors and performed more orderly dances. However, they were never able to produce normal distance encoding. Greater age, more experience following dances, additional practice with flying and foraging, or a combination of these factors could account for the improvements between E2_{Older Dancers} and E1_{First Dances naive} dances. Control bees improved by reducing distance range errors only when they were 20 days older (C2_{Older Dancers} versus C1_{First Dances}). Following experienced dancers before they first danced was sufficient for C1_{First Dances} bees to correctly order their dances with the lower number of directional errors typical of older, experienced bees.

Why should honey bees use social learning to improve their waggle dancing? Learning is a useful way to refine behaviors for local con-

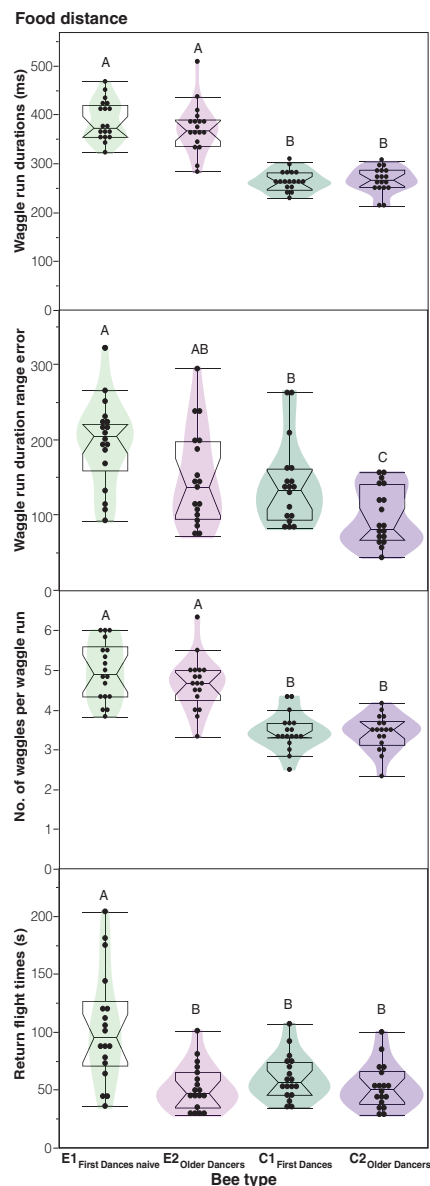


Fig. 2. Naive dancers that could not follow other dancers had disrupted distance encoding (waggle run durations and the number of waggles per waggle run) that persisted throughout their lifetimes. However, return flight times in experimental colonies significantly declined with experience. Different letters indicate significant differences.

ditions. We suggest that the distinct topologies of each colony's dance floor make it advantageous for novice dancers to learn from more experienced ones. Another possibility is that experienced dancers could transmit to nest-mates distance encodings that are based on local optic flow. Theoretically, distance encodings should be optimized according to the environment: the locations of food and the amount of optic flow that foragers experience when flying to this food. Because honey

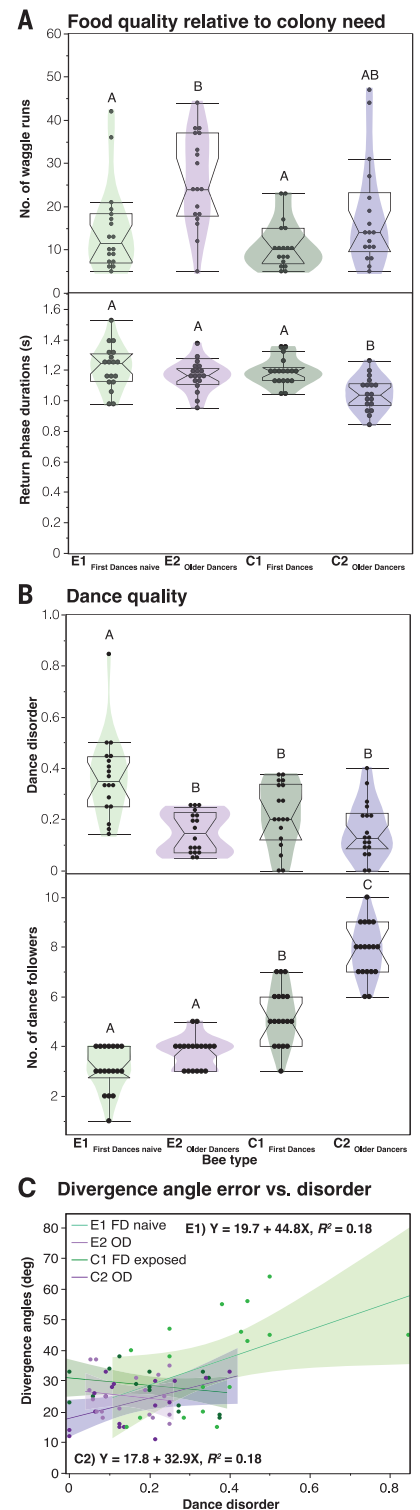


Fig. 3. Dance disorder was highest in naive first dancers and was positively correlated with angular error. Between groups, there were changes in (A) the communication of food quality and (B) dance quality and the number of dance followers (different letters indicate significant differences). (C) Directional error was positively correlated with dance disorder in E1_{First Dances naive} and C2_{Older Dancers} bees.

bee species can inhabit very different environments, distance encodings can be significantly different between species (14) and within species for *Apis florea* (22) and *Apis mellifera* (23). Given the imprecision inherent in waggle dances, the importance of these differences is not clear. Schürch *et al.* (24) compared the distance encodings of *A. mellifera* dancers in environments with different optic flow levels and found significant differences in the encoding line intercepts but not in the slopes. Our results indicate that we permanently altered distance encoding in our experimental colonies: After our treatment, novice dancers continued to make the same distance-encoding errors even near the end of their adult lives (25) despite decreasing their directional errors and dance disorder. Some aspects of the waggle dance can evidently be altered in young bees and are irreversible. Thus, we argue that the cultural modification and transmission of signals may be possible in social insects.

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ACKNOWLEDGMENTS

Funding: This work was supported by the CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. Additional funding was provided by the CAS 135 program (2017XTBG-T01) and the National Natural Science Foundation of China (31770420) to K.T. **Author contributions:** All authors contributed to the conceptualization and design of this research. S.D. and T.L. conducted the experiment, J.C.N. analyzed the data, and S.D., K.T., and J.C.N. contributed to the writing of the manuscript. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** Data are available at Zenodo (26). **License information:** Copyright © 2023 the authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original US government works. <https://www.science.org/about/science-licenses-journal-article-reuse>

SUPPLEMENTARY MATERIALS

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Submitted 28 July 2022; accepted 19 January 2023
10.1126/science.ade1702



Supplementary Materials for

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Science **379**, 1015 (2023)
DOI: [10.1126/science.ade1702](https://doi.org/10.1126/science.ade1702)

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Materials and Methods

Location

All 10 colonies (five control and five experimental) were housed at the bee apiary in the Southwest Center for Biological Diversity, Chinese Academy of Sciences (Kunming, China). Experiments were carried out from April-June of 2021 and 2022 when the temperature differences between day and night were moderate and therefore facilitated the survival of our young bee colonies.

Creating colonies

To create colonies with age-specific cohorts, combs with late stage (purple-eyed pupae) were removed from haphazardly selected healthy colonies and placed inside an incubator (PRX-250B, Ningbo Saifu Experimental Instrument Co., Ltd.) for 24 h (dark environment, 34 °C, and 75% relative humidity). As soon as they emerged, young bees were transferred to a two-frame observation hive with a new egg-laying queen to create experimental colonies. Upon creation, these experimental colonies (E) thus contained no eggs or brood, only the queen, 2800 newly emerged bees, and approximately a half comb of pollen and a half comb of honey.

Control colonies (C) consisted of approximately 2600 workers of all ages and had combs that contained similar amounts of stored honey and pollen as the experimental colonies. To create control colonies, we removed combs from a donor colony, placed them inside an observation colony, and provided a new laying queen. In each control colony, we marked 200 newly emerged bees with paint pens to create the first waggle dances in the control colonies C1_{First Dances} bees (workers that were exposed to and could follow waggle dances before they performed their first waggle dances) that controlled for the first waggle dances in the experimental colonies E1_{First Dances naive} bees (workers that had no opportunity to follow waggle dances before they performed their first, naive waggle dances).

First waggle dances with no prior experience

We continuously observed all colonies every day during daylight hours until we saw the first bees flying out to perform orientation flights and forage (on average, 9.0 ± 2.0 days old for E1_{First Dances naive} bees and 9.9 ± 1.0 days old for C1_{First Dances} bees). There was no significant difference in the age of first foraging between experimental and control colonies ($R^2_{\text{adj}}=0.06$, $F_{1,34}=3.21$, $P=0.08$). Although these early foraging ages are not typical, foragers can begin foraging at such an early age: 8-14 days old (27). We then immediately began training these foragers to a 55% w/v sucrose feeder placed 150 m from the colony. The locations were chosen so that a direct flight path from the colony to the feeder resulted in bees from each colony experiencing similar levels of optic flow, which is encoded to communicate distance in the waggle dance (21). Each feeder consisted of a 70 ml vial (8 cm high) inverted over a

circular plastic disk with 18 feeding grooves through which the sucrose could flow. After being filled with sucrose solution, the vial was inverted on the plastic disk and placed over a blue paper circle to help the bees return to the feeder once they had learned its location. Over 30 foragers could visit this feeder without crowding. We did not add scent to the feeders. As needed, we removed bees with an aspirator to reduce the number of bees and ensure that the feeder was not crowded.

To train bees, we carefully placed a glass vial at the entrance of the nest to trap the bees flying out and brought them to a feeder 150 m away, where they were released and began to imbibe sucrose. As they were feeding, we individually marked them with different paint pen colors (Edding 750). After the bees had visited the feeder a few times, they would waggle dance. All bees danced on their first day of foraging. We used a high-definition video camera (HDR-PJ790, Sony) and recorded the first waggle dance performances of five different bees per colony. A waggle dance performance is defined as a bee returning to the colony, waggling dancing, and then exiting the colony to forage again. These trained foragers did not follow each other's dances. Because there were no other waggle dancers in these colonies, their dances were “naive” in the sense that they did not have the opportunity to learn from other waggle dancers. For all foragers that we video recorded, we also measured how long it took them to fly back from the feeder to the nest immediately before they waggle danced (defined as return times with measurements coordinated with the hive and feeder observers via two-way radios).

Revisiting the same waggle dancers after they had 20 d of experience

We retrained the marked foragers whose first dances we had observed, 20 days later, to the same 150 m feeder locations and recorded their waggle dances to determine if their dancing (E2_{Older Dancers} and C2_{Older Dancers} bees) had changed. The workers were then, on average 29 and 30 days old in experimental and control colonies, respectively. Because *A. mellifera* adult workers live, on average, 31 days during the months of our experiment (25), we estimate that E2_{Older Dancers} and C2_{Older Dancers} bees had respectively reached 94% and 97% of their mean maximum adult lifespan.

We also video-recorded these waggle dancers and measured their return times (see above). We hypothesized that E2_{Older Dancers} dances, due to experience, would have increased precision and orderliness above that of E1_{First Dances naive} dances. In these 20 days between E1_{First Dances naive} and E2_{Older Dancers} dances, the feeder was not available, but we observed waggle dancing for natural food sources and E2_{Older Dancers} foragers therefore had the opportunity to follow the dances of other bees, forage for natural food sources, and waggle dance themselves. Based upon qualitative observations, there were no noticeable differences in overall colony dancing for nectar or pollen in E1_{First Dances naive}, E2_{Older Dancers}, C1_{First Dances}, or C2_{Older Dancers} phase colonies, perhaps because they were created with comparable levels of honey and pollen stores, had similar population sizes, and were kept at the same apiary. However, the E2_{Older Dancers} could

only follow other dancers of the same age with similar experience levels. In contrast, C2 Older Dancers could follow dancers of different ages with different experience levels.

Measuring the waggle dance

To analyze the waggle dance videos (recorded at 60 fps), we used Tracker software (V4.91). The researcher making these measurements was blinded to the treatment category and colony origin of the bee being observed. For each dance of every bee, we excluded the first waggle run because it is more variable (28) and analyzed the subsequent six waggle runs. We define a dance as a series of consecutive waggle runs and return phases during one visit of a forager inside the nest.

For each dance (Fig. 1), we measured: (1) the waggle run angle relative to gravity, (2) the divergence angle of the waggle dance (the maximum difference between waggle angles during six waggle runs), (3) the durations of waggle runs (defining the start of the waggle run by the start of dancer wing oscillations and the stop of the waggle run by the cessation of dancer wing oscillations)(28), (4) the waggle duration range error (the duration difference between longest and shortest waggle run within a dance), (5) waggle duration variance (coefficient of variance of the waggle durations), (6) the number waggles per waggle run (each waggle defined as one complete from right to left to right movement of the abdominal tip, Fig. 1A), (7) variance in the number of waggles per waggle run (coefficient of variance of the number of waggles per waggle run per dance), (8) return phase duration, (9) return phase duration variance (coefficient of variance), (10) the total number of waggle runs within a dance, and (11) the number of dance followers (each follower defined as a bee following a waggle dancer for ≥ 5 s) per waggle dance performance.

We also measured (12) the rate of return phase non-alternations, which we term “dance disorder”. After a dancer makes a waggle phase, it can make a return phase by turning either to its left (L) or its right (R). Dancers often alternate these return phases (i.e. L-R-L-R). A non-alternation return phase occurs when it makes two consecutive return phases in the same direction. For example, the pattern L-L-L-L would be counted as three return phase non-alternations out of four return phases (dance disorder proportion of 0.75) because we compare each return phase direction with the prior one. Tan et al. (29) called such non-alternating turns “disorder” and showed that foragers feeding on toxic nectar from the thunder god vine (*Tripterygium hypoglaucum*) produced significantly more non-alternation return phases than foragers feeding on non-toxic nectar. We therefore also define “disorder” as the number of non-alternating return phases and calculated the disorder proportion, the total number of return phase non-alternations divided by the total number of waggle runs within a dance.

The significance of dance disorder to dance followers is not clear. Followers may predict, to some degree, the direction in which a dancer will next turn, to assist their tracking, but this remains to be

determined. In hives with highly disoriented waggle dances (waggle runs pointing in all directions), followers eventually lost interest in these dancers and began foraging on their own (30). In the less extreme conditions of our experiment, followers should still be able to obtain largely correct directional bearings from E1 First Dances naive dances by averaging multiple waggle runs.

Statistical analyses

We used JMP Pro V16.1.0 to analyze our data. To test for differences in the age of first foraging between experimental and control colonies, we used Analysis of Variance (ANOVA) with colony type (experimental or control, a fixed nominal effect) as the independent variable and age of first foraging as a continuous dependent variable.

For the measurements shown in Table 1, we used Repeated Measures Mixed Models (REML algorithm) with colony type (experimental or control), bee ID (a random effect nested within colony type), time point (first dances or 20 days later, an ordinal variable), the interaction of colony type x time point, and colony as a random effect. Colony type and time point were fixed effects. Based upon inspection of model residuals, we log-transformed waggle durations, waggle duration range errors, and the number of waggle runs. To test for differences between the treatment groups within a dance on variance in waggle durations, the number of waggles per waggle run, and return phase durations, we calculated the coefficient of variation ($CV = \text{standard deviation} / \text{mean}$) and ran our models with these coefficients of variation. To make all corrected pairwise comparisons, we used Tukey Honestly Significant Difference (HSD) tests. We report the results of these tests in Table 1 and focus, in our results, on the significant pairwise differences. To test for correlations between divergence angles and disorder proportions per dance, we used linear regressions, one per bee type (E1 First Dances naive, E2 Older Dancers, C1 First Dances, and C2 Older Dancers).



Figure S1. Waggle dancer (w) and followers (f) on a natural comb dance floor. Note the irregular comb cells that vary between colonies and that the dancer must successfully negotiate while producing the complex waggle dance (see Fig. 1A for a full description of the waggle dance).

Name	Definition	Rationale
Experimental colony	A single cohort colony created from bees that are all 1-day old.	Experimental colony
E1 naive First Dances (E1_{FD naive})	The first waggle dances performed by bees (9.0±2.0 days old, mean±1 standard deviation) in an experimental colony. They are “naive” because these bees could not follow other dances before performing their first dance.	What do the first dances of foragers that have never danced before or followed other dances look like?
E2 Older Dancers (E2_{OD})	The waggle dances of E1 _{First Dances naive} bees when they are 29 days old and have more experience following other dancers and dancing.	Do the dances of E1 _{First Dances naive} bees change when they are 20 d older and have had experience dancing and following other dances?
Control colony	A multi-cohort colony consisting of bees of all ages.	Control colony
C1 First Dances (C1_{FD})	The first waggle dances performed by bees (9.9±1.0 days old) in a control colony (control for E1 _{First Dances naive}).	What do the first dances of foragers of foragers that have never danced before but were able to follow other dancers look like?
C2 Older Dancers (C2_{OD})	The waggle dances of C1 _{First Dances} bees when they are 30 days old (control for E2 _{Older Dancers}).	Do the dances of C1 _{First Dances} bees change when they are 20 d older and have had additional experience dancing and following other dances?

Table S1. Definitions of the different colonies and bee types. The rationale explains the main reason for observing the dances at each stage.

Video files may be downloaded or viewed at the following links. Downloading will usually enable the viewer to see the video at a higher quality.

Video S1. First waggle dance of a C1 bee (orange and green paint on thorax)

<https://drive.google.com/file/d/1EObbZDFw2pijA2SIYWSfsg8rWR5pRwIH/view?usp=sharing>

Video S2. First waggle dance of an E1 bee (green and purple paint on thorax)

<https://drive.google.com/file/d/13NT01HbrdAU4WpI-UVbT-4rGoSh1LzRh/view?usp=sharing>

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fiberoptic paths. Also, the nonlinearity in the fiberoptic loops means that photons can interact. Thus, photons can jump from one pulse to another through nonlinear interactions and redistribute into different modes. The photons can thereby thermalize just like a gas. The result is a highly controllable thermodynamic testbed for designing a negative-temperature heat engine that uses a photon gas as a working substance. For instance, the total size of the lattice can be increased or decreased with the variable coupler, thus increasing or decreasing the number of modes. The internal energy of the system can also be changed while keeping the number of modes constant.

The time-synthetic lattice is described by lattice band theory, which is analogous to the energy bands of a crystal lattice. The upper energy bound within each band is necessary to realize negative temperatures. In this system, negative temperatures are created just by adding more energy. For example, by increasing the intensity of the laser light that is injected to the loop system of Marques Muniz *et al.*, the energy of the system can be increased, which leads to a negative temperature. The variable coupler allows the abrupt doubling of the number of occupied modes to realize a sudden expansion of a photon gas. By contrast, if the lattice time difference between modes is resized slowly, then isentropic compression and expansion can be implemented, which are the building blocks of a heat engine. Throughout these processes, the negative temperature is stable, thus confuting the notion that negative temperatures are not practically useful (14).

As negative temperatures become realizable in accessible experimental contexts such as nonlinear optics, a rapid exploration of their impact can be expected, from the design of nanoscale superefficient engines (8) to quantum transport devices (14) to the generalization of the many-temperature distributions found in quantum simulators and computing (6). ■

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10.1126/science.adg7317

ANIMAL BEHAVIOR

Bees learn to dance

Experience yields precision in the waggle dance of honey bees

By Lars Chittka¹ and Natacha Rossi²

Many animals can guide or call other members of their group to a rich foraging site (1–3). By contrast, honey bees have a distinctive form of communication that allows them to send nestmates to the location of a food source by using symbols. The coordinates are encoded by intricate movements (the “dance”) on the vertical wax comb in the hive, using gravity and time as references. The motions are followed by recruits in the darkness of the hive, who subsequently decode the extracted flight vector information and follow the dancer’s instructions once outside (4). Like many of the elaborate behaviors of social insects, this communication system was thought to be innate. However, on page 1015 of this issue, Dong *et al.* (5) reveal that honey bees only deliver precise spatial information in their dances if they previously had the opportunity to attend dances by experienced role models—the communication system must in part be learnt socially.

After the discovery of a rich food source, honey bee (genus *Apis*) foragers can recruit nestmates by performing a figure-of-eight-shaped dance (consisting of a central “waggle run” followed by alternating left and right semicircles) on the vertical wax combs inside the hive, with followers touching the dancer’s abdomen with their antennae. The duration of the straight waggle run informs the others about the distance to the bounty. Direction of the target relative to the Sun is encoded in the angle of the waggle run, so that a waggle run straight up means “fly toward the Sun’s azimuth” and a waggle run at an angle 20° to the right of the vertical means “fly 20° to the right of the Sun’s azimuth” (4). The full dance circuit is repeated many times over to allow dance followers to average out variation of the display. There are indications that dance behavior is at least in part genetically encoded: All species of honey bees exhibit a form of this communication, and no other bee species do.

Moreover, subtle variations of the dance code within the genus are species specific, and the information contents are largely preprogrammed in that they are limited to information about location and quality and cannot easily incorporate new “words” (new symbols with new meanings) in the same way that human language can (6).

However, if the waggle dance was fully innate, young bees would display the dance correctly even if they had never witnessed the behavior. Dong *et al.* created bee colonies composed exclusively of newly emerged bees; without any guidance from tutors, these bees began displaying waggle dances at the typical age of 1 to 2 weeks after emergence from the pupae (7). But the location indications from such inexperienced bees were highly variable from one dance circuit to the next and consistently indicated distances longer than the bees had actually traveled. Recruits would have struggled to find the indicated location. As the immature bees gained experience over the coming 20 days, the variation of their location codes gradually reached normal levels. However, distance indications remained abnormally high for life, indicating that after a critical time window, adjustments through social learning are no longer possible (8). Bees from control colonies, which had exposure to dances of seasoned foragers before initiating their own, displayed none of these shortcomings.

Why does any element of the dance language have to be learnt if the end point of the learning is always a dance of the same pattern and precision? There are two possible scenarios—one is similar to human locomotion, whereby everyone has to learn to walk, but the outcome is predictable. The alternative scenario is that there might be flexibility in the outcome of learning (the dance patterns displayed) depending on the environmental conditions encountered by bees. This indicates the exciting possibility that the link between symbol and meaning could be learnt, as in human communication.

Could it be that what is socially learnt is not just the precise choreography, but the translation of the information provided by other bees’ dances into the actual coordinates of food sources subsequently encountered by the dance attendees? In

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support of this possibility, one species of honey bee was found to learn to read the distance code of another species, even though these two species normally encode distance differently (9). Bees' flight distance estimation is in part determined by the amount of contrast in the environment and thus differs between, for example, forests and steppes. Therefore, it is at least plausible that there might be subtly different, socially acquired local "cultures" of the dance language that depend on visual characteristics of the landscape or the spatial distribution of food sources (10).

The study of Dong *et al.* adds to the growing evidence that complex behaviors are seldom entirely innate. For example, although the regularity and optimality of the honey bee comb construction were regarded by Darwin as "the most wonderful of all known instincts" [(11), p. 235], it turns out that how workers build comb is affected by the comb structures that they experienced when young (12). Even specialist bee species, supposedly innately tied to certain species of flowers, must learn to manipulate these flowers (13).

Some scholars assume that instinct is by default the ancestral (or primitive) state and that learning is more advanced. The opposite is more rarely considered: Individual learning might be at the root of some behavior innovations that are now partly innate. Bees can learn even relatively arbitrary behaviors, such as string pulling or ball rolling, by observing skilled conspecifics (14). It is therefore plausible that some of their most advanced behavioral innovations (including elements of the dance language) might have emerged at least in part by individual innovation and subsequent social learning, becoming instinctual later in evolutionary time (14, 15). Therefore, the observed flexibility of species-specific behavior might simply reflect the ancestral condition. ■

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10.1126/science.adg6020

CELL BIOLOGY

Watching biomolecules stride in real time

A noninvasive imaging technique tracks the motion of single biomolecules in live cells

By **Jinyu Fei** and **Ruobo Zhou**

A long-sought goal for scientists is to directly watch motions and interactions of all individual biomolecules within a cell, which would substantially increase our understanding of life processes at the molecular level. On pages 1004 and 1010 of this issue, Wolff *et al.* (1) and Deguchi *et al.* (2), respectively, take us one step closer to this goal. They report an improved version of MINFLUX, a nanoscope concept introduced 6 years ago (3), that increases the spatiotemporal resolutions of light microscopy to nanometer and millisecond scales. They apply this technique to study the molecular mechanisms of kinesin walking on microtubules under unprecedented physiologically relevant conditions.

In the journey to view objects inside cells with light microscopes, great successes have been made for visualizing cell organelles, which are typically 1 to 10 μm across. However, visualizing the crowded, single proteins inside cells with light microscopy is challenged by the diffraction limit of visible light (4). Light microscopes can distinguish between two fluorescent objects divided by a lateral distance of approximately half the wavelength of light used to image the objects, and hence the smallest feature size that light microscopes can resolve is ~ 250 nm, whereas proteins are only ~ 5 nm. A group of superresolution imaging methods, collectively called fluorescence nanoscopy, have recently been developed that circumvent the diffraction limit and have pushed the spatial resolution down to 10 to 30 nm (4).

There are two main categories of fluorescence nanoscopy approaches. The first category, such as stimulated emission depletion microscopy (STED), surpassed the light diffraction limit with patterned illumination, in which an additional coaxial donut-shaped depletion laser beam is added to the point-scanning confocal excitation laser beam to inhibit fluorescence emission everywhere other than at the very center of the diffraction-limited illumination region. This allows

the center region, which is much smaller than the diffraction-limited region, to emit fluorescence. The second category, such as stochastic optical reconstruction microscopy (STORM) and photo-activated localization microscopy (PALM), is based on single-molecule localization, in which a superresolution image is constructed from a camera-recorded series of time-separated image frames, each of which contains only a sparse set of fluorescent molecules so that the centroid positions of these molecules can be individually localized by using two-dimensional (2D) Gaussian fitting to find the peak position of each molecule's fluorescence intensity profile. The precision of this peak finding (localization) is inversely proportional to the square root of the photon number collected for building the single-molecule fluorescence intensity profile (5). The spatial resolution of these traditional camera-based localization nanoscopy approaches is also limited to the maximum photon number that a fluorophore can emit per localization, which is an intrinsic property of fluorophores.

In 2017, MINFLUX was introduced to push the spatial resolution down to 2 to 3 nm, enabling true molecular-scale fluorescence imaging (3). In contrast to traditional camera-based localization that uses the fluorescence intensity maximum, MINFLUX shifted a donut-shaped illumination spot over an area of a few hundred nanometers around each fluorescent molecule to localize these molecules by using the fluorescence intensity minima. This requires 10 to 100 times fewer photons compared with that of camera-based localization to achieve the same localization precision. The unprecedented spatial resolution of MINFLUX is achieved by combining the strengths from both categories of nanoscopy approaches: Using photo-switchable dyes to excite only a small subset of dyes at a time for single-molecule localization, as used in STORM and PALM, while using a point-scanning donut-shaped beam as used in STED to localize the fluorescence intensity minimum. MINFLUX has been successfully used to visualize cellular ultrastructures—such as the multiprotein mitochondrial contact site and cristae organizing system (MICOS) (6), the nuclear pore complex (7), and neuro-

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