Title: Individual Learning Phenotypes Drive Collective Cognition

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One Sentence Summary: Attentive individuals more strongly influence collective foraging decisions by focusing on and recruiting to preferred food locations.

ABSTRACT: Collective cognition emerges from individuals responding to local information to make decisions. Animals vary in how they learn to pay attention to important information, which has unknown consequences on collective behavior. We tested whether honey bee colonies comprised of individuals that differ in their capacity to focus their attention would choose to continue to visit learned, familiar food locations or switch to novel food locations. We found that colonies of focused bees preferred familiar food locations, while colonies of unfocused bees visited all sites equally. In mixed colonies, both types of bees preferred the familiar food location. Focused bees recruit unfocused nestmates to their preferred foraging location by dancing more intensely. Our results reveal that attention-focusing abilities of individuals drives emergent collective behavior.

Main Text:

Collective cognition allows animal groups to more effectively respond to a rapidly changing environment compared to their individual counterparts, such as schools of fish avoiding predators (*1*), flocks of birds migrating across the Earth (*2*), or honey bee colonies finding and visiting millions of flowers (*3*). Individuals in groups utilize local information to quickly adjust to ecological changes by implicitly or explicitly communicating information with group members to form collective behavior (*4*–*6*). Collective cognition emerges from interactions between individuals that vary in their cognitive abilities (*3*, *4*). Variation in the cognitive task of attention can shape critical

individual behaviors, such as effectively cueing in on the correct prey or mate (*7*), and can have severe consequences on learning and productivity in humans (*8*–*10*). However, little is known about how individual variation in attention may influence emergent collective behavior.

We therefore tested how individual differences in learning to focus attention scales to shape collective foraging behavior in honey bees by utilizing a learning behavior called latent inhibition (LI) (*11*). Latent inhibition is a form of non-associative learning related to the ability to ignore familiar, unrewarding stimuli to pay attention to novel, important stimuli (*12*). Specifically, after exposure to an odor that is not followed by reward, some forager bees take longer to learn about this now 'familiar' odor, relative to a novel odor, when both types of odor are later paired with reward, or "high LI". Other bees that learn both familiar and novel odors quickly are "low LI". Moreover, differences in expression of LI correlate to foraging specializations. LI has been mostly studied in vertebrates (*12*–*16*). However, honey bees (*Apis meillifera*) also exhibit (*17*, *18*) and show heritable variation in LI (*11*). Scout bees, who search for novel food sources, show high LI whereas recruited bees that forage at known food locations show low LI (*19*). To uncover how individual variation in learning to pay attention impacts collective behavior, we investigated how an individual's latent inhibition (LI) may scale to influence collective foraging behavior in honey bee colonies.

The reproductive queens and haploid males (drones) of a honey bee colony exhibit variation in LI (*11*, *18*). This allowed us to select for distinct high and low LI phenotypes to generate lines from queens singly inseminated with sperm from like-performing drones to produce workers that exhibit similar LI to their parents (*20*). We

70 then created colonies composed of age-matched single cohorts of only low, only high,
71 50/50 mixed high and low LI workers, as well as age-matched non-selected control
72 bees. In semi-natural foraging conditions, we counted the number of forager visits, first
73 visits, and revisits to the familiar or novel food locations. To explore the mechanisms
74 underlying how individual variation in LI affects collective foraging, we quantified the
75 round recruitment dance activity in mixed colonies while foraging.

76 To ensure that selected bees in different social environments exhibited the
77 predicted heritable LI phenotype, we evaluated the LI score of foragers from selected
78 lines that spent their adult life in either their natal colony or a control colony. Foragers
79 retained the expected LI based on the LI of their parents, regardless of whether they
80 were housed with same or with variable learning phenotypes (GLM: $\chi^2 = 6.54$, df=1,
81 p=0.011, Fig. 1). We did not detect an effect of the identity of the colony in which the
82 bees were housed on LI phenotype ($\chi^2 = 3.766$, df=2, p=0.152, Fig. 1).
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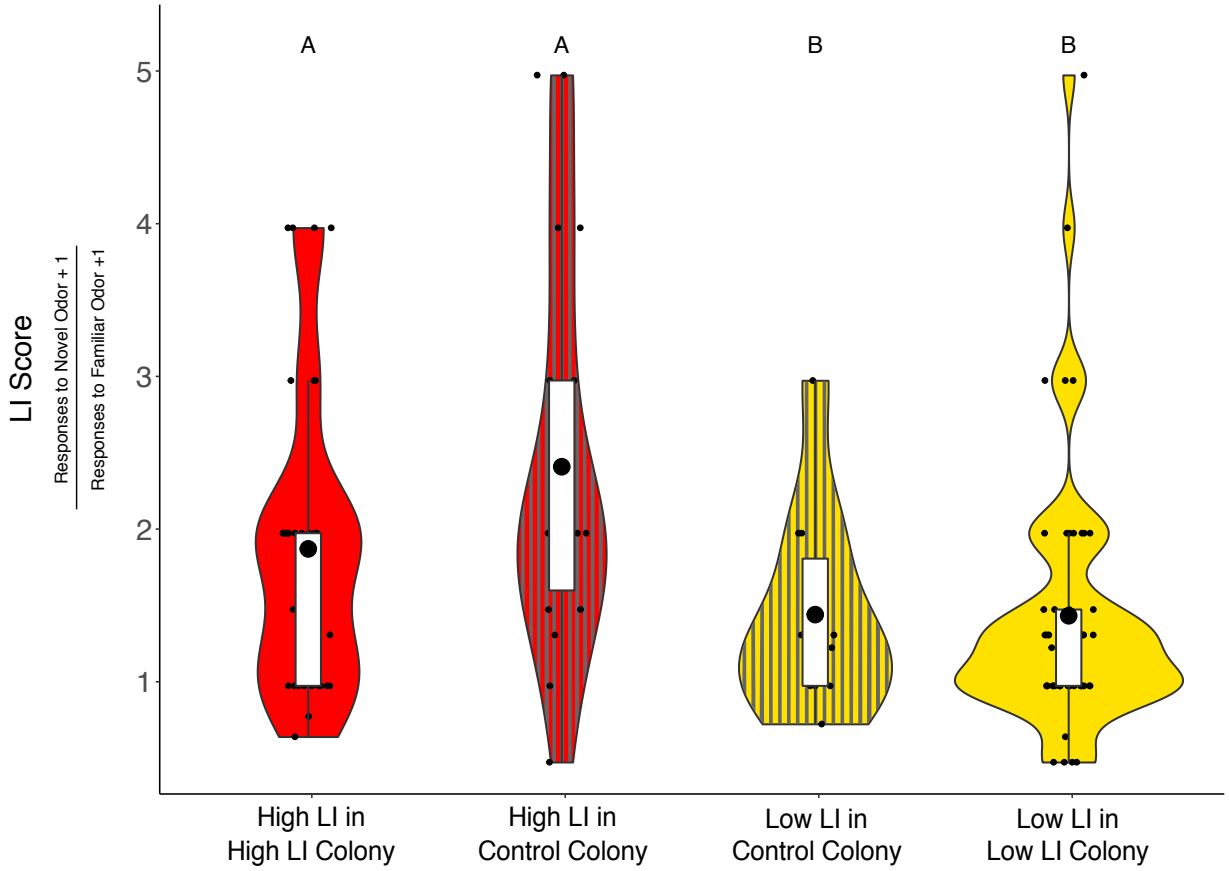

Figure 1: Social environment does not alter expression of genetically selected latent inhibition. *LI scores of individuals from high LI lines that spent their adult life either in high LI only colonies (red, n=36) or in a control colony with a variety of LI phenotypes from an open mated queen (red with gray vertical lines, n=18); individuals from low LI lines that spent their adult life either in low LI only colonies (yellow, n=52) or in control colonies (yellow with gray vertical lines, n=10). Different letters above boxes indicate statistically significant differences according GLM comparison (p<0.05). In this and subsequent figures, the large black dot is the mean, the white box is the interquartile range (IQR), whiskers extend to 1.5*IQR, and the small points beyond the whiskers are outliers. Color shaded areas show the*

distribution of the data. Additionally, here and in all following figures, yellow are low LI colonies and individuals, gray are control colonies and individuals, and red are high LI colonies and individuals.

To determine how the learning phenotypes influenced colony-level foraging behavior, we placed small single-cohort age-matched colonies into a flight cage and monitored foraging activity to familiar or novel feeders (Fig. 2A). To evaluate the collective feeder choice, we recorded the number of visits to each feeder by bees from each selected line according to the color of paint on the bees' thorax. We further marked bees with a feeder-specific color on their abdomen when they visited the feeder for the first time to determine if bees revisited that feeder.

Colony learning phenotype strongly influenced overall number of visits to the food locations (GLM: $\chi^2 = 1270$, df = 3, $p < 0.0001$, Fig. 2B). Foraging in the high, low, and control colonies was largely performed by bees revisiting the feeders, while mixed colonies had more new visitors to the feeders (GLM, $\chi^2 = 22.32$, df =3, $p<0.0001$, Fig. 2C, table). A colony's LI phenotype composition also determined its preference between the novel and familiar feeders, with high LI and mixed colonies preferring the familiar and low and control colonies showing no preference (GLM: Feeder*Colony χ^2 =473.64, df=3, $p<0.0001$; Fig. 2D). Revisits to feeders drive foraging for all colonies (Fig. 2E: Colony*Feeder χ^2 =53.67, $p<0.0001$). However, new foragers in the mixed colonies that visited the novel feeder were less likely to return to it compared to foragers who visited the novel feeders in other colonies (Table S6).

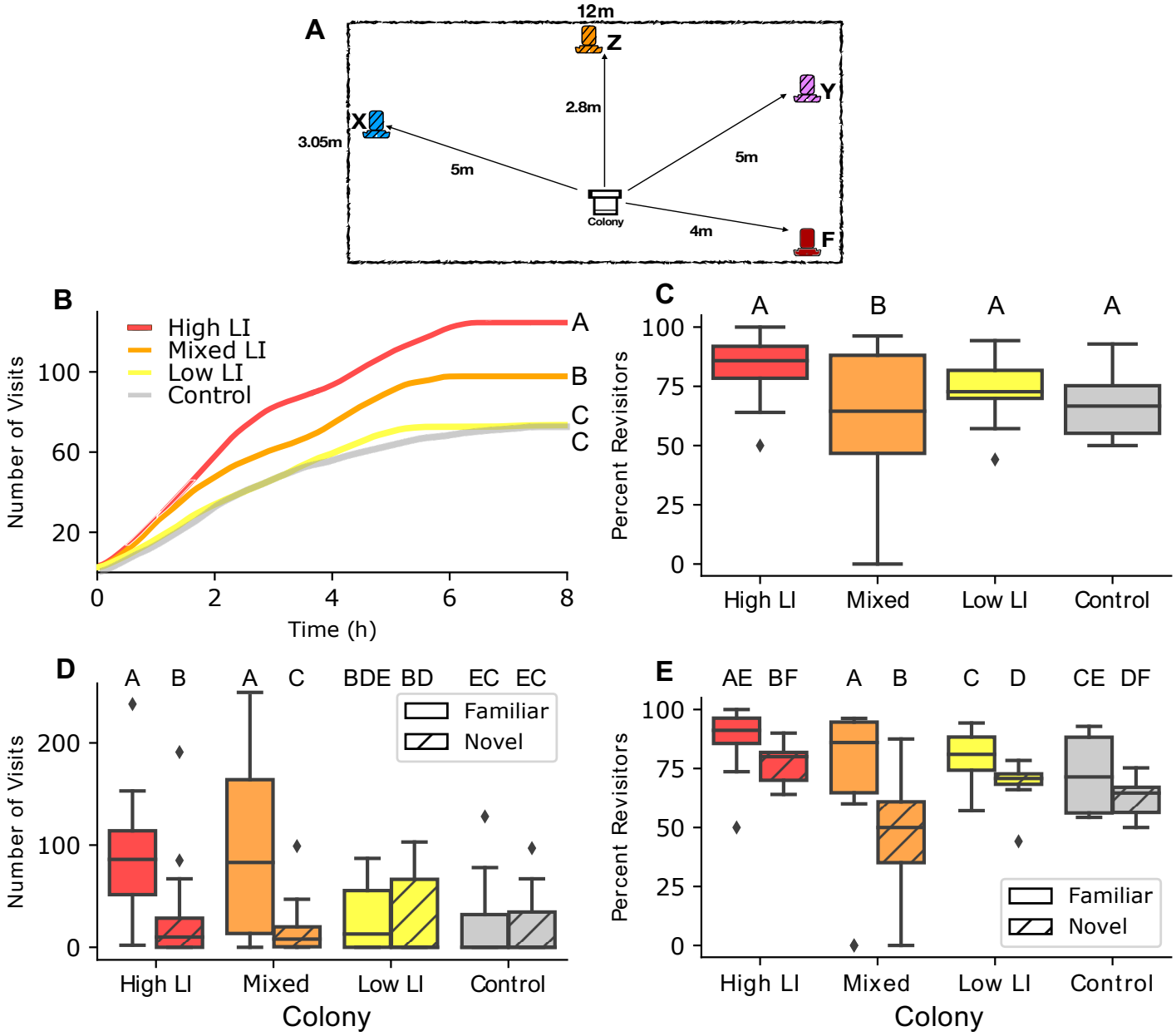

Figure 2: Colonies constructed from different genetic lines selected for high or low latent inhibition exhibited differences in collective foraging behavior. *(A) The experimental set up illustrating the location of feeders in relation to the location of the colony (center, white) within the experimental arena (large rectangle) (See table S2 for*

feeder placement and odors) (B) Cumulative number of visits of all bees (bees from selected lines and controls) to all feeders over time by colony type. Different letters to the right of the lines indicate statistically significant differences according to a post hoc test (p<0.05) (See fig S1 for individual visitation). (C) Percent of re-visits out of the total number of visits to all feeders by colony type. Here and in all following panels, different letters above boxes indicate statistically significant differences according to a post hoc test (p<0.05, table S4). (D) Number of all visits to the familiar feeder (solid boxes) and a novel feeder (hatched boxes) for each type of colony, when both novel and familiar feeders were presented simultaneously (days 2-4) (for post-hoc results see table S5). (E) Percent of re-visits out of the total number of visits to either the familiar or the novel feeder by type of colony when both novel and familiar feeders were presented simultaneously (days 2-4) (for post-hoc results see table S6). N=24 colonies, 6 colonies per colony type, 6172 total visits.

To determine why the mixed colonies showed a preference for the familiar feeder (Fig. 2D), we examined how individual lines visited each feeder (Fig. 3). In 2017, we tested mixed colonies placed in a flight cage. In 2018, we reselected lines and then placed mixed colonies into two-frame observation hives to evaluate recruitment dances along with visitation to the feeders in the flight cages. We found that there was a significant year effect (table S7), likely due to reselection and different hive placement. We therefore statistically analyzed each year separately to focus on the within-year variation between the selected lines.

Low LI and control individuals shift their preference to the familiar feeder when mixed with high LI bees. In 2017, we found a significant interaction between the selected line and which feeder foragers visited (GLM: χ^2 =7.79, df=2, p=0.02; Fig. 3A). Although low LI and control colonies did not show a preference to a novel or familiar feeder when they had a uniform colony composition (Fig. 2E), when mixed with high LI individuals, low LI and control individuals exhibited a preference to the familiar feeder (Fig. 3A, table S8). High LI individuals showed a preference to familiar feeders just as colonies comprised of only high LI individuals did (Fig. 2E). We found a significant interaction between selected line and feeder again in 2018 (GLM: χ^2 =85.27, df=2, p<0.0001; Fig. 3B), with low LI and control individuals showing preference to the familiar feeder over the novel feeder (Fig. 3B, table S9) similar to high LI individuals preferring the familiar feeders.

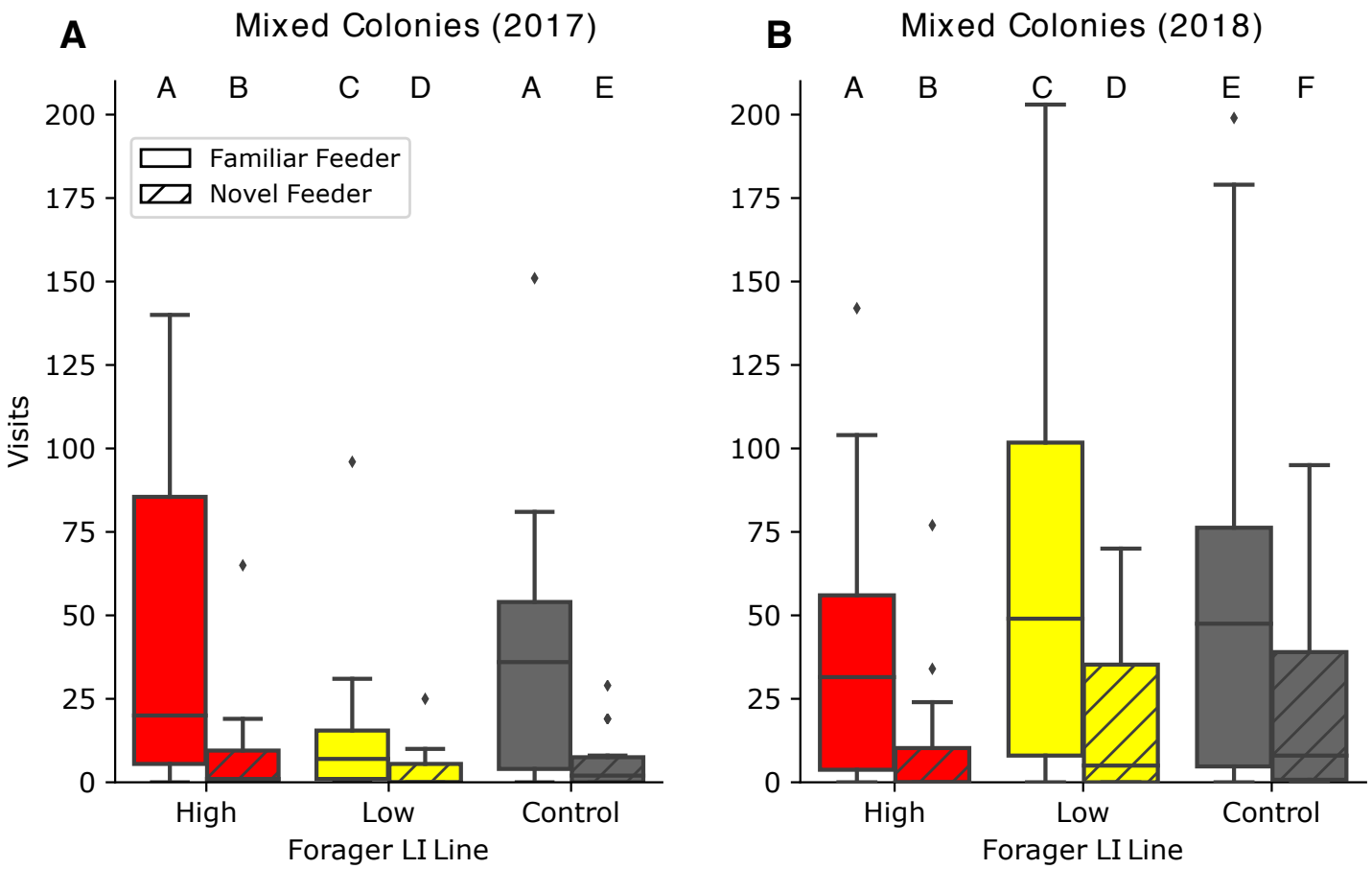

Figure 3: Visits of individuals from different genetically selected lines when in a mixed colony. *Daily visits to the familiar and novel feeders by individual bees in mixed colonies from low LI parents (yellow), high LI parents (red) or open mated queens (grey) in (A) 2017, N=6 mixed colonies, 2347 overall visits and (B) in mixed colonies from lines that were re-selected in 2018, N=6 colonies, 6272 overall visits. In both years, low LI and control bees switched to prefer the familiar feeder.*

175 To uncover the behavioral mechanisms that underlie why low LI and control bees
176 switch feeder preference when housed with high LI bees, we evaluated how each line
177 performed the round dance, the modified waggle dance used for recruitment to food at
178 short distances (*21*). Individuals from the lines differed in their likelihood to perform a
179 round dance (Chi-square test: χ^2=26.61, df=2, p<0.0001; Fig. 4B). Low LI individuals
180 were significantly more likely to perform a dance compared to high Li individuals
181 (pairwise chi-square test: p=0.0001) and controls (pairwise chi-squared test: p=0.004).
182 High LI individuals were just as likely to perform a dance as controls (p=0.36).
183 Individuals differed in their likelihood to follow a dance based on their selected line (Chi-
184 square test: χ^2=28.26, df=2, p<0.0001; Fig. 4B). Low LI individuals were significantly
185 more likely to follow a dance compared to high LI bees (pairwise chi-square test:
186 p<0.0001) and controls (p<0.003). High LI and control individuals were equally likely to
187 follow a dance (p=0.240).
188 Although the high LI individuals danced less often, high LI dances had
189 significantly more followers compared to low and control bees (Chi-square test: χ^2=
190 13.93, df=2, p<0.001; Fig. 4C). Low LI bees performed more dances that had no
191 followers compared to high LI and control dances. Low LI individuals spent more time
192 dancing; however, they attracted fewer followers than high and control dancers,
193 indicated by the significant interaction between the LI of the dancer and dance duration
194 when predicting the number of followers (GLMM: χ^2= 6.42, df=2, p=0.04; Fig. 4E). The
195 relative attraction of dances of high LI bees could be due to the intensity of the dance.
196 High LI bees performed more turns per second during their dances (ANOVA: χ^2=12.8,
197 df=2, p=0.002; Fig. 4F).

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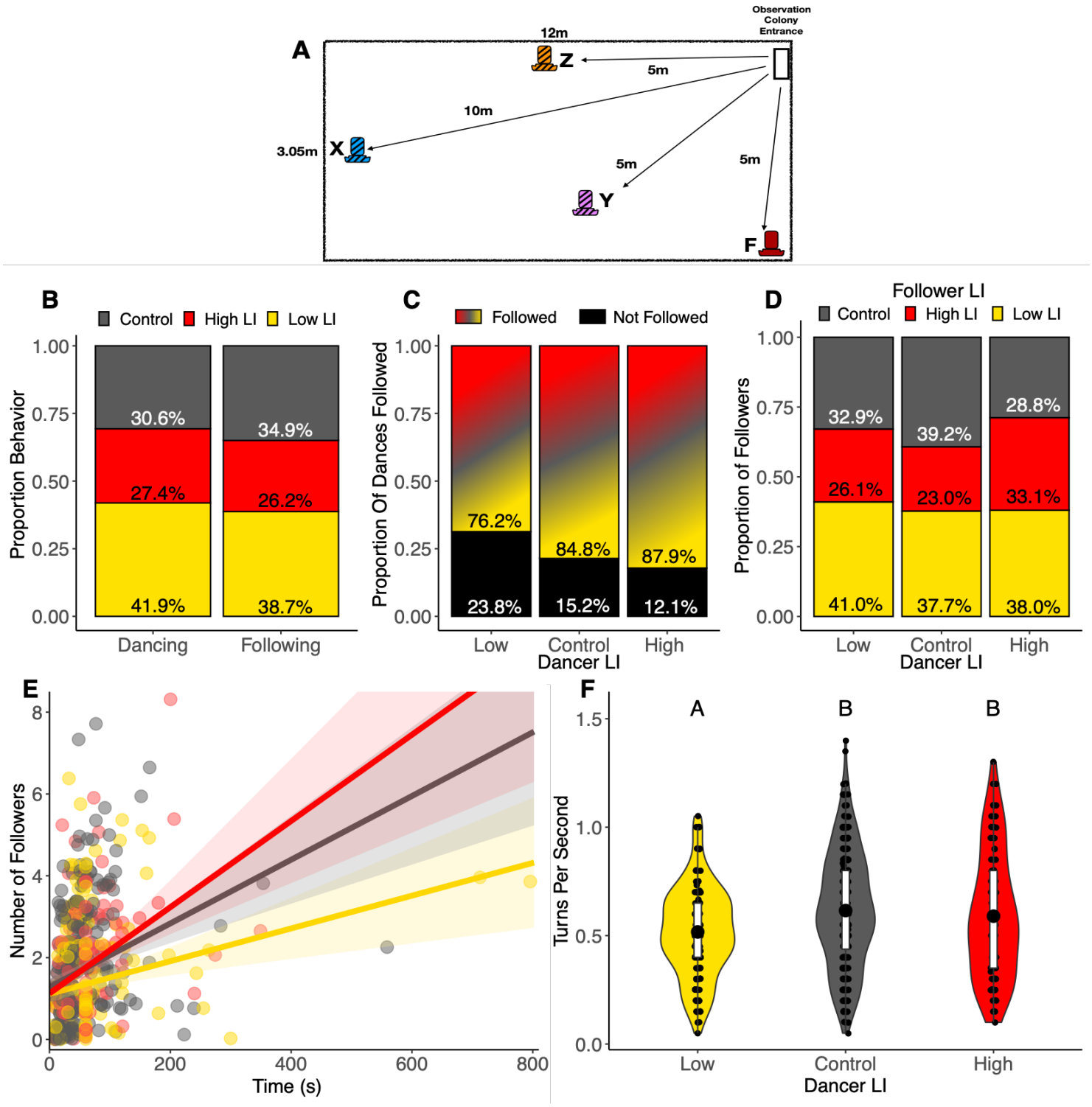
A
Observation Colony Entrance
12m
Z
5m
10m
3.05m
X
5m
5m
Y
F
B
Control
High LI
Low LI
Proportion Behavior
30.6%
27.4%
41.9%
34.9%
26.2%
38.7%
Dancing
Following
C
Followed
Not Followed
Proportion Of Dances Followed
76.2%
23.8%
84.8%
15.2%
87.9%
12.1%
Low
Control
High
Dancer LI
D
Follower LI
Control
High LI
Low LI
Proportion of Followers
32.9%
26.1%
41.0%
39.2%
23.0%
37.7%
28.8%
33.1%
38.0%
Low
Control
High
Dancer LI
E
Number of Followers
Time (s)
F
A
B
B
Turns Per Second
Low
Control
High
Dancer LI

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Figure 4: Recruitment dances facilitate integration of information from different genetically selected lines. *(A) The experimental set up illustrating the location of feeders in relation to the location of the colony entrance (top right, white) within the experimental arena (large rectangle). (See SI Table 2 for feeder placement and odors) (B) Proportion of dances (N=667) or follows (N=1201) across 6 colonies performed by bees from each line, relative to their abundance in the mixed colony (350 high, 350 low, 700 control). We accounted for the difference in abundance of each selected line by dividing the number of observed control dancers by 2 before calculating these proportions. (C) Proportion of dances performed per LI line type that were either followed by at least one individual (colored) or not followed by any other bees (black). (D) Proportion of dances by LI line type that were followed (from panel B) broken down by LI of the follower. (E) Relationship between number of followers and duration of a dance by line. Point and line colors indicate LI of dancer. Best fit line represents the GLM, shaded area represents the 95% confidence interval. (F) Rate of turns per second in a dance by line.*

By combining techniques from experimental psychology and behavioral ecology, we have developed a system for investigating how variation in individual learning behavior drives collective cognition. We demonstrate that a laboratory-selected heritable learning behavior with natural individual variation scales to shape the collective performance of honey bee colonies on quasi-natural foraging tasks. In the lab, high LI bees learn to ignore familiar odors that they experienced without reinforcement, while

223 readily learning novel odors. One interpretation of reduced learning to a familiar,
224 unrewarding, stimulus is that pre-exposure reduces attention to, and thus associability
225 of, that stimulus (*22*, *23*). In this interpretation, latent inhibition allows animals to focus
226 attention on important information (*15*, *24*, *25*). Our observations of field behavior of low
227 and high LI individuals and colonies are consistent with this interpretation, whereby high
228 LI individuals have stronger attention capacities to food compared to low LI individuals.
229 Once high LI individuals have found a food location, they continue to revisit it,
230 'attending' more strongly to reinforced feeders over new ones. The increased impact of
231 the resource on these bees could translate into stronger, more vigorous dances. In
232 contrast, low LI individuals learn and visit both known and new feeders equally, dividing
233 their attention across resources and acting more like generalist foragers. In mixed
234 colonies, this broadened attention by low LI individuals may therefore make them the
235 perfect audience for the high LI dancers, driving them to prefer feeders that high LI
236 individuals preferentially visit. Under natural conditions, where queens mate with many
237 different drones, most colonies would possess both types of learners, perhaps more
238 closely resembling our mixed colonies (*26*). We propose that this diversity of 'attention'
239 aspect of individual cognitive phenotypes may enhance the overall efficacy with which a
240 colony finds and exploits resources (*27*). In summary, our work indicates that individual
241 cognition scales to shape the collective cognition of animals solving critical ecologically
242 relevant tasks.
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Author Contributions: CNC, TM, JG, NPW, BHS conceived of and helped design the study. CNC, NJL, and TM analyzed the data. CNC and CO created the genetically selected lines and CO maintained them. CNC carried out data collection and wrote the first draft of the manuscript. CNC, TM, NJL, NPW, BHS discussed results. All authors commented on the manuscript. **Competing Interests:** The authors declare no competing financial interests **Data Availability:** Data will be available on FigShare and code will be available on Github upon publication.

SUPPLEMENTAL MATERIALS

Materials and Methods

Tables S1-S9

Fig. S1

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