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Heritable Cognitive Phenotypes Influence Appetitive Learning but not Extinction in Honey Bees

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Abstract

Learning and attention allow animals to better navigate complex environments. While foraging, honey bees (*Apis mellifera* L.) learn several aspects of their foraging environment, such as color and odor of flowers, which likely begins to happen before they evaluate the quality of the food. If bees begin to evaluate quality before they taste food, and then learn the food is depleted, this may create a conflict in what the bee learns and remembers. Individual honey bees differ in their sensitivity to information, thus creating variation in how they learn or do not learn certain environmental stimuli. For example, foraging honey bees exhibit differences in latent inhibition (LI), a learning process through which regular encounter with a stimulus without a consequence such as food can later reduce conditioning to that stimulus. Here, we test whether bees from distinct selected LI genotypes learn differently if reinforced via just antennae or via both antennae + proboscis. We also evaluate whether learned information goes extinct at different rates in these distinct LI genetic lines. We find that high LI bees learned significantly better when they were reinforced both antenna + proboscis, while low LI and control bees learned similarly with the two reinforcement pathways. We also find no differences in the acquisition and extinction of learned information in high LI and low LI bees. Our work provides insight into how underlying cognition may influence how honey bees learn and value information, which may lead to differences in how individuals and colonies make foraging decisions.

Learning and memory formation allows animals to efficiently navigate through their environments, forage for food, and select mates. In fact, many significant ecological mechanisms, such as aposematic coloration, depends on an animal’s cognitive abilities to observe and learn the colors of prey that made them sick (Dukas 2013). The study of cognitive ecology has shed light on the need to understand how cognition, including learning and memory, may play an important role in the ecology and evolution of animals (Dukas 2002). This is especially important in the context of collective behavior. In social groups, animals work together synergistically to accomplish critical tasks, like foraging for enough food for all individuals. The variation that individuals exhibit while learning about their environment may lead to differences in how collective animal groups accomplish tasks (Sumpter 2010, Cook et al. 2020).

In forming a mental construct of their environment, animals must not only learn what predicts important events, they must also learn what does not, and thereafter avoid the latter types of stimuli. Latent inhibition (LI) is a learning process through which regular encounter with a stimulus without a consequence, such as food, reduce an animal’s later conditioning to that stimulus (Lubow 1965, Shettleworth 2010). Many animals exhibit latent inhibition (LI), including fish and tadpoles learning predator versus nonpredator cues (Ferrari and Chivers 2009, Lubow and Weiner 2010, Mitchell et al. 2011). Fruit flies, crayfish, and honey bees (*Apis mellifera* L.) also exhibit individual variation in expression of LI (Abramson and Bitterman 1986; Chandra et al. 2000, 2010; Lubow and Weiner 2010; Cook et al. 2018; Lemanski et al. 2019; Cook et al. 2020; Smith and Cook 2020a). LI is hypothesized to facilitate attention to relevant information. Individuals that exhibit high LI also exhibit increased attention to the important stimuli, allowing them to learn more effectively (Lubow 1989). Latent inhibition may then mediate the process of animals paying attention to important information likely by habituating to the stimulus prior to that stimulus becoming associated with food.
As honey bees are foraging, they learn many aspects of their environment, including food location, floral odors, and how to navigate back to their colony using landmarks (Carr-Markell and Robinson 2014). Foragers often learn about a resource that becomes depleted and may have to switch foraging locations. Cognitively, some individuals may be able to switch quickly as that information goes extinct, whereas others may continue to visit the location for a longer time even though it may no longer be profitable (Ben-Shahar et al. 2000, Mosqueiro et al. 2017). LI is known to play a role in important divisions of labor in honey bee foraging. Bees who are scouting for new food sources tend to exhibit higher LI than bees that are recruited to these found food location (Cook et al. 2018). Pollen foragers, who respond to lower concentrations of sucrose (Pankiw and Page 2000), exhibit high LI compared with nectar foragers (Latshaw and Smith 2005). LI is heritable, which allows for the selection of reproductive queens and drones to create workers of known LI (Chandra et al. 2000). Foragers that are selected for high LI tend to visit familiar food locations, whereas foragers that are selected for low LI visit novel and familiar food locations equally (Cook et al. 2020). Genetically, LI is associated with an area on the genome that is associated with pollen versus nectar preference of foragers (Hunt et al. 1995, Chandra et al. 2001, Smith and Cook 2020b). Especially for social groups, cognition may play a significant role in individual and collective food choices.

Here, we evaluate whether bees with high or low LI genetic lines learn differently based on the reinforcement pathway as they learn and remember odor information, as well as how that information goes extinct. Using selected high and low learning lines, we first evaluated how honey bees from these different genetic lines learn when they are reinforced with just their antenna with a stronger reinforcement versus their antenna and proboscis with a weaker reinforcement. We predicted that high LI bees, who are more sensitive the lack of reinforcement, will learn better when reinforced with both antenna and proboscis, and will therefore learn less well with just antennal reinforcement. We then tested how quickly learned responses went extinct when bees from the selected lines no longer received reinforcement. We predicted that when the food reinforcement was removed, the association between an odor and a food reward would extinguish more quickly for the high LI bees compared with the low LI bees, again because high LI individuals are more sensitive to lack of reinforcement. Overall, this work helps us further understand how animals may learn about food sources to make effective foraging decisions.

Materials and Methods

Creation of LI Lines
To obtain queen honey bees for testing, we grafted 40 queens from 8 genetically distinct colonies. Successfully reared queens were placed in queenless ‘queen bank’ colonies for 10 d for nurse bees to care for them, after which queens were reproductively mature and ready for LI testing and insemination. To obtain drones, we placed mesh in front of the entrance of a colony and collected drones as they returned to their colonies from failed mating flights, which typically occurred in the late afternoon. When drones successfully mate, they die (Winston 1991). This ensures a higher likelihood that they were fertile. Drones were then placed into small cages and placed into queenless colonies for temporary storage and care from workers for testing the next day.

Conditioned Odor Stimulus Preparation
We prepared two different odors, used to test queens and drones as either novel or familiar. To prepare the odors, we pipetted 3 µl of pure odor, either 1-hexanol or 2-octanone (Sigma Aldrich, onto a 0.5 × 4-cm strip of filter paper (Whatman, VWR), then placed the scented filter paper into 3-in. long glass tuberculin syringe tubes (Sigma Aldrich). To prevent dissipation of scent, we used parafilm to cover the open ends of the syringe before use.
LI Testing of Queens and Drones Using Proboscis Extension Reflex

To test the LI of queens and drones, we followed typical LI testing as outlined in Cook et al. (2020), Cook et al. (2019), and Chandra et al. (2001). Briefly, we retrieved queens and drones from temporary storage in the queenless colonies. To strap in bees to harnesses, we removed bees from their boxes and placed them into plastic straws and strapped them down with Scotch tape. Scotch tape is less sticky and prevents damage to bees (Chandra et al. 2001, Cook et al. 2020). We do not chill queens and drones to minimize likelihood of damage to reproductive capabilities. Queens and drones were able to acclimate to harnesses for 1 h in a dark cabinet. We then tested whether bees were motivated by sucrose by touching a 0.4-µl droplet of 1M sucrose solution touched just to their antennae and they were not allowed to eat. Bees that did not respond were removed from the experiment (Wright et al. 2007, Chandra et al. 2010, Cook et al. 2018). To familiarize bees to an odor, we placed 16 bees onto an automated apparatus where bees were exposed to a 4-s puff of odor (1-octanone or 2-hexanol, Sigma Aldrich) every 5 min 40 times. The entire familiarization process took 285 m. After familiarization, bees rested for 15 min in the dark cabinet. We then used the proboscis extension reflex (PER) to test the ability of bees to learn the familiar compared with a novel odor. Familiarization is a process by which animals experience repeated stimuli during a fixed duration. During this process, no evaluation of habituation, defined as reduced responses to the stimuli occurs (Oakes 2010), although habituation is likely occurring to facilitate LI. To perform PER, we placed a single bee into a testing arena, allowed them to rest for 20 s, then puffed a scented airstream at the bee with the familiar (X) or the novel (A) odor, pseudorandomized as AXXAXXX or XAXAXXX, for 4 s (Smith et al. 1991, Fernandez et al. 2009, Bazhenov et al. 2013). Both the novel and the familiar odors were rewarded; the last second was paired with 0.4 µl of 1.5 M sucrose solution using a Gilmont Syringe (Cole Parmer) by touching sucrose droplet to each antenna until the proboscis was extended, then bees were allowed to drink the droplet. We classified a positive response as extending the proboscis within the first 3 s of odor exposure, before antennae were touched with sucrose (Chandra et al. 2000, Ferguson et al. 2001, Cobey et al. 2013). For more information on LI scores from each queen and drone that created a colony, see Cook et al. 2020).

Using responses during PER, we calculated LI score as follows (Cook et al. 2018):

\[
\frac{\text{No. responses to novel odor (A) + 1}}{\text{No. responses to familiar odor (X) + 1}} = \frac{\text{No. responses to novel odor (A) + 1}}{\text{No. responses to familiar odor (X) + 1}}
\]

We used instrumental insemination to inseminate a single queen with sperm from a single drone (Harbo 1986, Cobey et al. 2013). We inseminated low LI queens with low LI drones and high LI queens with high LI drones, as determined using the calculated LI score (see supplemental information from Cook et al. 2020). Bees that scored 1.33 or higher were considered high LI, whereas low LI bees scored below this threshold (Cook et al. 2018), and we chose individuals with the more extreme high and low scores for insemination (Cook et al. 2020). We then slowly introduced queens to small queenless colonies, where the queens produced workers for a month before we began to use them. We produced 10 high and 10 low colonies using these methods, and they were monitored weekly for supersedeure and queen cells were destroyed (Cobey et al. 2013). Selection and insemination took place April to May 2018 at the Honey Bee Research Lab at Arizona State University. Five of each line survived to 2019, of which we used three of each colony for these experiments.

Raising Colonies of High and Low LI

All experiments were conducted between March 2019 and August 2019. For ease of testing, we prepared small colonies of only high and only low workers. To prepare these colonies, we removed frames of capped brood from three selected colonies from each line and placed them into incubators set at 34°C for 24 h. All newly emerged bees were marked with originating colony and line-specific colors, then placed into small numbered nucleus colonies of all high LI or all low LI workers and a nonselected queen. These marked workers were
allowed to freely forage and were collected as incoming foragers, usually around 21 d. Control bees came from nonselected colonies (Cook et al. 2020).

Forager Collection
We collected bees from each colony using ventilated glass scintillation vials as they returned back from foraging trips (Wright et al. 2007). We avoided collecting foragers returning with pollen as they have different LI scores other foragers (Latshaw and Smith 2005). We typically collected 12 bees and tested 8, as some bees may not respond to the concentrations of sucrose used in these experiments. Bees in vials were then placed into insulated containers with cool packs to prevent bees from overheating during transportation back to the lab. We then placed them into an ice/water slurry bath and monitored them until they were immobilized, approximately 2–3 min. We then placed them into harnesses, allowed them to acclimate, and tested their responsiveness to 0.5 M sucrose, as outlined above and in Smith and Burden 2014. We used a lower concentration of sucrose as to test for minimum sucrose responsiveness, and we used a lower concentration during this testing phase as higher sucrose concentrations are related to enhanced learning and memory (Annau and Kamin 1961).

Associative Conditioning Bees Using Antenna Only Versus Antenna ± Proboscis Reinforcement Pathways
To evaluate how bees from selected LI learning lines respond to different methods of reinforcement via antennal or antennae and proboscis reinforcement pathways, we trained bees to two different odors (1-hexanol or 2-octanone) by touching the sucrose food reinforcement either just to the antennae or to the antennae and then the proboscis. Testing took place in a Plexiglas arena with a vacuum system to evacuate the scented air. A single bee was placed into the arena and allowed to acclimate for 20 s. A button was then pressed on a programmable logic controller (PLC, DL05, or DL105 model; AutomationDirect), which began a 4 s countdown. After 4 s, air began to pass through the odor-containing glass syringe that was directed at the bee, which lasted a total of 4 s. After 3 s of odor exposure, a noise was emitted from the PLC, signifying to the tester to reinforce the bee, so that bee associated the odor with the food reinforcement. If the odor was A, the bee was conditioned with 0.4 µl of a strong 2.5 M sucrose reinforcement touched just to their antennae, and the bee was not allowed to consume the droplet. We chose two different concentrations because we wanted to keep the bee motivated and continuing to extend her proboscis with only touching her antennae using a stronger 2.5M reinforcement. If the odor was X, the bee was conditioned with 0.4 µl a weaker 1 M sucrose reinforcement, touched to the antennae and then to proboscis and allowed to consume the entire droplet (Table 1; Wright et al. 2007). We used a Gilson syringe to precisely measure the reinforcement droplets. Bees then rested in the area for 30 s. Each conditioning trial lasted 55 s. The tested bee was removed, and the next bee was placed into the arena, and the process was repeated. 8 bees were tested at a time; as such, the inter-trial interval—the time in between each bee’s test—was 8 min. We recorded a positive response when the bee extended her proboscis during the first 3 s of odor exposure. For a video of these methods, see (Smith and Burden 2014). Bees were tested six times with each odor/reinforcement combination in a pseudorandom order: AXAXAXAXAX or XAXAXAXAXA. We tested 30 high LI bees from three different colonies, 29 low LI bees from three different colonies, and 25 control bees that came from two nonselected colonies.

Table 1. Reinforcement methods

<table>
<thead>
<tr>
<th>Antennae</th>
<th>Antennae + Proboscis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 µl of 2.5 M sucrose</td>
<td>0.4 µl of 1 M sucrose</td>
</tr>
<tr>
<td>Only touched to both antennae</td>
<td>Touched to both antennae then proboscis</td>
</tr>
<tr>
<td>Odor A</td>
<td>Odor X</td>
</tr>
</tbody>
</table>
Bees that were reinforced with food to just their antennae were reinforced with a strong sucrose solution but were not allowed to feed. Bees reinforced with antennae + proboscis were reinforced with a weaker sucrose solution and then allowed to feed.

After conditioning, bees were placed back into the dark cabinet for 15 min, then were then tested with one test trial of each odor that included no reward. We tested them by following the same PER protocol but did not provide the sucrose reward, then evaluated whether bees responded within the first 3 s of the odor exposure. We tested 26 high LI bees from three different colonies, 15 low LI bees from three different colonies, and 25 control bees that came from two nonselected colonies.

Acquisition and Extinction of Conditioned Information
We used the PER assay to condition foragers from each line to a single odor to evaluate whether bees from the selected lines learned a single odor differently. We collected foragers from the high- and low-nucleus colonies we created, then used the PER protocol to condition bees to respond to one odor, 2-octanone, over 5 trials using 1.5 M sucrose, touched to both the antennae and proboscis, which are typical PER methods. We then tested the bees with a single unreinforced test trial to ensure they learned.

To test how quickly conditioned information went extinct for forager honey bees, we used an unrewarded assay similar to the PER assay outlined above. After conditioning the foragers, they rested for 15 min in the dark resting location. We then assessed how quickly bees would stop responding to the conditioned odor by using the PER protocol, except the bees received no reward during the last second of the odor exposure. We did this over 15 trials. If bees extended their proboscis during the first 3 s of odor exposure, it was scored as 1, that the bee was retaining the learned response. If the bee did not extend its proboscis during those 3 s of odor exposure, then it was recorded as a 0, indicating the learned response was going extinct. We tested 36 control bees from two different unselected colonies, 29 low LI bees from three different colonies, and 18 high LI bees from three different colonies.

Statistical Methods
To evaluate both reinforcement pathway, acquisition, and extinction, we performed logistic regressions using a generalized linear model (GLM). In all models, the PER in the first trial and the last trial was our response variable, which was 0 for no learned response and 1 for a learned response. Learning trial (ordered: 1 < 6), genetic learning line (high LI, low LI, control), and reinforcement type (antennae or antennae + proboscis) were our predictor variables. We used a Wald type II $\chi^2$ analysis of variance (ANOVA) test to explore the overall main and interaction effects in the GLM. We then used a Tukey post-hoc test to further probe the significant interactions. We performed the same analysis separately for the single test trials for each odor. For evaluating acquisition and extinction, our predictor variables were trial and genetic learning line. To find the best model for all analyses, we used a backward selection approach, where we started with the most complex model including all fixed effects and interactions, then removed any insignificant interactions. All analyses were done in R (R Core Team 2020) and RStudio (Rstudio Team 2019) using the lme4 (Bates et al. 2014), emmeans (Lenth et al. 2018), and dplyr (Wickham et al. 2019) packages, and graphs were made using the ggplot2 (Wickham 2009) package.

Results
Genetic Lines Learn Differently With Antennal Versus Proboscis Reinforcement Pathway
We tested the effect of reinforcement pathway on the learning of selected LI lines. We found that LI, reinforcement method, and trial did not interact to significantly predict PER responses, indicating that within reinforcement methods, bees from the LI lines learned similarly across trials ($\chi^2 = 1.48, P = 0.47$). After removing the nonsignificant three-way interaction, we found that the overall interaction between LI and
reinforcement significantly predicts learning (Table 2; Logistic Regression: LI * Reinforcement Method: \( \chi^2 = 7.07, P = 0.029 \); Fig. 1). Overall, high LI bees were significantly more likely to learn when reinforced via the antenna + proboscis method compared with the antennae-only reinforcement (Tukey posthoc test, proboscis-antennae: Estimate = 2.09, Z = 3.01, \( P = 0.003 \); Fig. 1A). There was no significant difference in learning via the two different reinforcement methods for the low LI bees (Tukey posthoc test, proboscis-antennae: Estimate = 0.06, Z = 0.11, \( P = 0.91 \); Fig. 1B), or control bees (Tukey posthoc test, proboscis-antennae: Estimate = 1.14, Z = 1.76, \( P = 0.07 \); Fig. 1C). Another major predictor of learning was reinforcement method interacting with trial (\( \chi^2 = 19.96, P < 0.01 \)). Specifically, antennae-only reinforcement showed fewer responses across trials but still learned (Tukey posthoc test: Estimate = 1.69, Z = 4.32, \( P < 0.01 \)) compared with learning when reinforced via antennae + proboscis across trials (Estimate = 4.53, Z = 8.04, \( P < 0.01 \)). Finally, we found that LI and trial did not interact to significantly predict PER responses, as the lines learned similarly across the trials overall (\( \chi^2 = 3.77, P = 0.15 \); Supp Fig. 1 [online only]).

Table 2. The overall GLM table illustrating the interactions and main effects that shape the learning response

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Overall GLM</th>
<th>df</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LI</td>
<td>4.89</td>
<td>2</td>
<td>0.086</td>
</tr>
<tr>
<td>Reinforcement</td>
<td>13.37</td>
<td>1</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Trial</td>
<td>117.92</td>
<td>1</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>LI:Reinforcement</td>
<td>7.07</td>
<td>2</td>
<td>0.029*</td>
</tr>
<tr>
<td>LI:Trial</td>
<td>3.77</td>
<td>2</td>
<td>0.15</td>
</tr>
<tr>
<td>Trial:Reinforcement</td>
<td>19.96</td>
<td>1</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Trial:Reinforcement:LI</td>
<td>1.48</td>
<td>2</td>
<td>0.47</td>
</tr>
</tbody>
</table>

The full GLM is PER Responses (binomial) ~ Trial (1,6) + LI (high, low, control) + Reinforcement (Antennae or Antennae + Proboscis) + Trial*LI + LI*Reinforcement + Trial*Reinforcement+ Trial*Reinforcement*LI. Values remained the same at these decimal places after the three-way-interaction was removed. An asterisk indicates significance at alpha <0.05.

Fig. 1. Learning in high LI, low LI, and control foragers when reinforced with antenna or antenna + proboscis. (A) High LI bees (\( n = 30 \)) learned significantly better when reinforced with both their antennae and proboscis and did not learn as well when just reinforced with a sucrose reward to their antennae. (B) Low LI bees (\( n = 29 \)) showed no significant difference in learning with different reinforcement methods. (C) Control bees (\( n = 25 \)) showed no significant difference in learning with different reinforcement methods. An asterisk * indicates significant differences from others at \( P < 0.05 \).
Antenna ± Proboscis Reinforcement Resulted in Better 15-min Short-Term Memory
We then tested whether the reinforcement method and LI lead to bees having better 15-min short-term memory. We found that reinforcement method drove the responses to the single-exposure unrewarded test trials (Logistic Regression: $\chi^2 = 66.27, P < 0.001$; Fig. 2). Bees that were reinforced to antenna + proboscis responded significantly more during the test phase compared with bees who were reinforced to just their antennae, indicating that on the short term they retained the information significantly better ($Z = -5.88, P < 0.001$). Genetic line had no effect on test responses ($\chi^2 = 0.44, P = 0.8$).

Fig. 2. Testing of 15-min short-term memory formation after two reinforcement methods (antenna and antenna + proboscis) using the PER responses. Test trials were performed on all high ($n = 26$), low ($n = 15$), and control ($n = 25$) bees 15 min after the end of the PER assay. Genetic line had no effect on test responses. (A) Bees from all tested genetic lines retained the learned response better when reinforced with both antennae + proboscis compared to just antenna. (B) The distribution of responses shows bees reinforced with proboscis + antennae exhibit better short-term memory compared to bees reinforced with antenna only. The dark gray being proboscis + antennae reinforcement and the light gray is antenna only reinforcement. Different letters indicate significance at $P < 0.05$.

Acquisition of Odor Information and Extinction of That Learned Response Remain Consistent Across Learning Lines
There was no significant difference in associative learning across the different lines ($\chi^2 = 3.35, P = 0.18$; Fig. 3A). There was also no significant difference among the different genetic lines regarding extinction of their responses to the learned odor over 15 unreinforced trials ($\chi^2 = 5.52, P = 0.06$; Fig. 3B).
Fig. 3. Acquisition and Extinction curves for each genetically selected learning line. (A) Genetic lines showed no significant differences in their associative learning responses across the five conditioning trials. (B) Genetic lines were equal in extinction of the learned information. Solid lines with dark gray dots are control bees ($n = 36$), small dashes with gray dots are high LI ($n = 18$) bees, large dashes with light gray dots are low LI bees ($n = 29$). The same bees tested for the acquisition of odor were later used for testing the extinction of their learned information.

Discussion

Here, we found that bees from different cognitive genetic lines learn differently when they are reinforced by either just antennae or antennae and proboscis. Bees from the line selected for high LI learn odor information best when they are able to sense the food with their antennae and consume the food with their proboscis. Bees from the low LI lines readily learn information in both contexts: when the sucrose solution is presented to just to their antennae and when provided to their antennae and proboscis. Foragers from these different learning lines exhibited no difference in associative learning or extinction of a learned response. Our results indicate that high LI bees require tasting of food to effectively learn information associated with that food source. These differences in learning may have nonlinear effects for how colonies learn about foraging locations (Lemanski et al. 2019, Cook et al. 2020).

High LI foragers must fully evaluate the information presented before they learn and remember it (Figs. 1 and 2). This is also similar to differences in high LI and low LI bees learn about food sources as they forage. In semi-natural field conditions, high LI foragers focus on the first available food source and rarely switch to a novel food source, whereas low LI foragers will forage at both familiar and novel food sources equally (Cook et al. 2020). High LI foragers then communicate the higher perceived value more intensely than the low LI bees (Cook et al. 2020). The ability to focus attention on important information may be mediated by their inhibition to other food sources (Lubow 1989), especially if the initial food source is consistently available. There is no difference in sucrose response thresholds between high LI and low LI foragers (Bennett et al. 2020), indicating that increased focus is determined by more than perception of high-value rewards. Individual cognitive differences seem to translate into how individuals perceive and communicate about the information they acquire (Couzin 2009).

How bees learn may also influence the choices they make when selecting where to forage or for what to forage for. For example, bees that forage for pollen also tend to exhibit high LI, whereas bees that forage for nectar exhibited lower LI (Latshaw and Smith 2005). Pollen foragers also perform better on many cognitive tasks, such as associative learning, reversal learning (Drezner-Levy et al. 2009), memory tests (Wright et al. 2007), and exhibit better learning of tactile stimuli (Scheiner et al. 2001a, b), compared with nectar foragers. Wright et al.
2007 found that nectar foragers equally well when reinforced using their antennae compared with antennae + proboscis. The current study investigated the nectar foragers with high LI and low LI. These learning differences may be mediated by how they value the information they acquire while learning, leading to differences in how quickly they learn or how long they remember that information. Specifically, pollen foragers respond to lower concentrations of sucrose (Pankiw and Page 2000), potentially allowing them to value information associated with rewards readily. LI is associated with PLN2, a quantitative trait loci (Chandra et al. 2001) that is also associated with food preference and the concentration of nectar collected by nectar foragers (Hunt et al. 1995), which indicates that the cognitive characteristics of LI may influence the same gene networks that drive food preference. We tested returning foragers that were not carrying pollen and therefore were presumably nectar foragers, which may account for low LI bees learning information associated with both reward pathways similarly. Gene networks that shape behavior are complex (Sinha et al. 2020), and several of these networks that influence cognition and perception may drive behaviors that together lead to broader phenotypes like foraging preferences.

Extinction is the disrupted or inhibited responses to a conditioned association after reinforcement is no longer presented (Eisenhardt 2012). How rapidly learned associations extinguish in bees is associated with how quickly they are learned, and a reduction in speed of extinction is associated with aging and reduced ability to learn (Münch et al. 2010). In our results, we found no differences in how high or low LI foragers formed conditioned associations, or how quickly they went extinct (Fig. 3), which does not support our initial hypothesis. Although high LI bees are slow to become conditioned to an already familiar, unreinforced odor, they will quickly learn when odors that once signaled food no longer do. The learned extinction response also occurred quickly in all tested genetic lines (Fig. 3), showing that both are equally capable of learning when odors are no longer associated with incoming food. Just like conditioning, extinction consists of new learned response rather than an ‘unlearning’ of previously learned information (Eisenhardt and Menzel 2007). Both lines learn the conditioned association and the extinction response equally well.

Learning allow animals adapt to a changing environment. As honey bees forage, they may need to abandon a resources as it becomes depleted. Some individuals may be more likely to give up on a resource compared with others. This may also depend on the proportion of scouts, who explore the landscape for new food, and recruits, who learn about food locations from scout bees and exploit these known locations (Biesmeijer and Vries 2001). Theoretical models indicate that the level of persistence in the performance of each task, i.e., how likely an individual was to return to that known resource, dramatically changes the food collection performance of the colony (Mosqueiro et al. 2017). Both high LI and low LI bees extinguish learned information equally well. However, LI highly correlates with another cognitive behavior: reversal learning, where individuals are able to reverse their learning when one odor switches from being reinforced to unreinforced (odor exposure with no reward), and another odor that was previously unreinforced is reinforced. Individuals that exhibit high LI also exhibit slower reversal learning (Chandra et al. 2000). As high LI bees seem to be more attentive and focused to learned information, they may be less likely to switch as resources deplete. To this end, our work indicates that high LI bees may be more scrutinous about a food source if they cannot fully evaluate using both their antennae and proboscis, suggesting when they do learn about information, they have a more difficult time divesting in the information they’ve already learned. High LI bees then seem to be the more persistent foragers, investing strongly only when they have fully vetted the information, and communicating that accordingly.

The genetic component of many of these cognitive traits indicate that there is likely a selective advantage for variation in cognitive performance, especially in social groups. Diversity of traits in a collective group often leads to a more robust response to environmental changes (Jones et al. 2004, Nonacs and Kapheim 2007, Mattila et al. 2008). As such, cognitive differences likely provide similar robustness to rapid environmental shifts. Here, we demonstrate that honey bees from cognitively selected genetic backgrounds (high and low LI foragers) vary in
how they learn when reinforced via different sensory modalities (antennae only or antennae + proboscis). However, both genetic lines showed no difference in the acquisition and extinction of their learning. This further emphasizes the need to explore how individual cognitive behaviors may scale to collective outcomes, as well as the use of insects to explore cognition at multiple levels of organization.

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