RESEARCH ARTICLE

Underlying mechanisms and ecological context of variation in exploratory behavior of the Argentine ant, *Linepithema humile*

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ABSTRACT

Uncovering how and why animals explore their environment is fundamental for understanding population dynamics, the spread of invasive species, species interactions, etc. In social animals, individuals within a group can vary in their exploratory behavior, and the behavioral composition of the group can determine its collective success. Workers of the invasive Argentine ant (*Linepithema humile*) exhibit individual variation in exploratory behavior, which affects the colony's collective nest selection behavior. Here, we examine the mechanisms underlying this behavioral variation in exploratory behavior and determine its implications for the ecology of this species. We first establish that individual variation in exploratory behavior is repeatable and consistent across situations. We then show a relationship between exploratory behavior and the expression of genes that have been previously linked with other behaviors in social insects. Specifically, we found a negative relationship between exploratory behavior and the expression of the *foraging* (*Lhfor*) gene. Finally, we determine how colonies allocate exploratory individuals in natural conditions. We found that ants from inside the nest are the least exploratory individuals, whereas workers on newly formed foraging trails are the most exploratory individuals. Furthermore, we found temporal differences throughout the year: in early-mid spring, when new resources emerge, workers are more exploratory than at the end of winter, potentially allowing the colony to find and exploit new resources. These findings reveal the importance of individual variation in behavior for the ecology of social animals.

KEY WORDS: Collective behavior, Exploration, *Foraging*, Gene expression, Invasive species, Behavioral ecology, Personality, Repeatability

INTRODUCTION

Exploratory behavior is fundamental for how animals interact with their environment. Animals explore new environments when they disperse from their natal habitat (Stamps, 2001), expand their home range (Onen and Hanski, 2006) and search for new resources (Kramer and Weary, 1991). For example, exploratory individuals of invasive species can be responsible for expanding the invaded range (Cote et al., 2011; Fogarty et al., 2011). Despite the advantages of exploring for new resources, there are many associated costs,

including spending valuable energy during the search process (Stamps et al., 2005), encountering novel predators (Eliassen et al., 2007; Kramer and Weary, 1991) and ending up in worse habitat than the one from which the animal departed (Stamps, 2001).

Social animals can benefit from the discovery associated with exploration with minimal costs because individuals in social groups vary in their behavior. Thus, non-exploratory group members may benefit from new information obtained by risk-taking exploratory group members, without paying the costs associated with obtaining the new information. For example, in bird flocks, 'scroungers' access resources found by 'producers' (Aplin and Morand-Ferron, 2017; Giraldeau et al., 1994; Katsnelson et al., 2008). Individuals in a group could be competing over resources, and so studies of producer–scrounger dynamics often focus on competition rather than the potential mutual benefits (Beauchamp and Giraldeau, 1996; Koops and Giraldeau, 1996). In social insects, selection acts at the level of the colony, and so if certain individuals can enhance the colony's ability to obtain resources, the entire colony benefits. For example, in honey bees, certain foragers act as 'scouts' (Biesmeijer and de Vries, 2001), who are more likely than other foragers to locate new resources (Liang et al., 2012). When the scouts find new resources, they recruit other foragers to the location of these resources (von Frisch, 1967), thus improving overall colony foraging success, especially in patchy environments (Anderson, 2001; Beekman and Bin Lew, 2008; Dechaume-Moncharmont et al., 2005; Donaldson-Matasci and Dornhaus, 2012; Dornhaus and Chittka, 2004).

Animals vary consistently in their behavior (Sih et al., 2004) and these consistent behavioral differences have long been studied in social insects (Beshers and Fewell, 2001; Gordon, 1996; Jaisson et al., 1988; Jandt et al., 2014; Oster and Wilson, 1978). Work on social insects has focused primarily on differences among individuals in which tasks they perform, such as foraging, nursing, nest maintenance, etc. However, there is also variation in the way that each individual performs a task; for example, some individuals may be diligent in their performance of any task, whereas others perform tasks only intermittently and spend much of their time resting (Charbonneau and Dornhaus, 2015; Pinter-Wollman et al., 2012; Robson and Traniello, 1999). This behavioral variation among individual workers can have great implications on the collective behavior and success of the colony (Hui and Pinter-Wollman, 2014; Pinter-Wollman, 2012; Pruitt and Riechert, 2011). Yet, only little is known about the mechanisms that underlie the behavioral differences among individuals and about the broad ecological implications of these behavioral differences (Beekman and Jordan, 2017).

One potential mechanism that can underlie variation in behavior is genetic variation, specifically, variation in the expression of particular genes, which allows for both flexibility and persistence (Bell and Aubin-Horth, 2010; LeBoeuf and Grozinger, 2014). Behavioral genomics has uncovered gene candidates that control

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social behavior (Robinson et al., 2005; Smith et al., 2008; Zayed and Robinson, 2012). For example, the honeybee model system has provided extraordinary insights into the genes whose expression regulates the division of labor between foraging (Ben-Shahar et al., 2002), scouting (Liang et al., 2012), nursing (Whitfield et al., 2003) and defense (Alaux and Robinson, 2007). One well studied gene is the *foraging* gene, which was first identified in *Drosophila melanogaster* as the gene that distinguishes between 'rover' and 'sitter' foraging strategies at the larval stage (Debelle et al., 1989). The expression of *foraging* positively associates with movement during larval foraging in *D. melanogaster* (Osborne et al., 1997). In Hymenoptera, this gene positively associates with foraging behavior in honeybees (Ben-Shahar et al., 2002) and negatively associates with foraging behavior in harvester ants (Ingram et al., 2005), although expression patterns can vary with exposure to light throughout the day (Ingram et al., 2016).

The Argentine ant [*Linepithema humile* (Mayr 1868)] has invaded ecosystems throughout the world with remarkable success (Suarez et al., 2001). Workers of *L. humile* vary in their exploratory behavior, and a group's composition of exploratory behavior determines its ability to select a suitable nest site (Hui and Pinter-Wollman, 2014). Colonies of *L. humile* occupy more than one nest site (i.e. are polydomous) and expand the number of nest sites they occupy during the spring, when resources are abundant (Heller and Gordon, 2006). *Linepithema humile* establish persistent foraging trails to long-lasting food sources and in between nests, and they form smaller trails to new ephemeral resources (Flanegan et al., 2013). Uncovering the mechanisms that underlie the collective exploration of *L. humile* can help predict where they will spread to next and aid mitigation of further spread.

Here, we ask whether individual variation in exploratory behavior is persistent and whether colonies of *L. humile* allocate exploratory individuals to where they are most needed and during the times of year when they are most beneficial to the colony. First, we examine whether individual workers maintain the same exploratory behavior over days and across different assays for exploratory behavior. We then ask whether there is a relationship between exploratory behavior and the expression of *foraging*. We predict that because foraging behavior requires extensive movements outside the nest and the red harvester ant (*Pogonomyrmex barbatus*) ortholog *Pbfor* is downregulated in foragers (Ingram et al., 2005), we will find a negative relationship between exploratory behavior and the expression of *Lhfor*, the *L. humile* ortholog of the *foraging* gene. Finally, we ask where and when exploratory individuals can be found in a natural setting. If colonies allocate exploratory individuals to where they are most needed, we expect to find the least exploratory individuals

inside the nest and the most exploratory individuals on newly formed foraging trails. We further expect to find more exploratory individuals in the spring, when colonies expand their range, compared with in the winter.

MATERIALS AND METHODS
Exploratory behavior
Exploratory behavior of individual ants was quantified using an eight-arm maze with spices at the end of each arm, following the methods in Hui and Pinter-Wollman (2014) and Modlmeier and Foitzik (2011) (Fig. 1A). An ant was placed in the center chamber of the maze for 5 min and we recorded the total number of visits it made to any spice as its exploratory behavior. A visit was defined, as in the studies cited above, as an ant moving at least one body length (10% of the length of the arm) into the maze arm leading to a spice. At the end of each trial, we cleaned the apparatus with ethanol and replaced spices that were touched by the ant.

To examine whether exploratory behavior is a persistent trait, we collected 66 *L. humile* ants from a colony on the University of California, San Diego (UCSD) campus in October 2014. We housed the ants in individual containers, and tested their exploratory behavior three times, on three consecutive days, once each day. We determined the repeatability of exploratory behavior using the intraclass correlation coefficient (ICC), which quantifies similarity of observations within a certain group – in this study, repeated observations of a single individual (Bell et al., 2009). The more similar the observations within a group, the greater the ICC. We used the 'ICC' package in R to calculate this measure (Wolak et al., 2012).

To determine whether the eight-armed maze assay reliably captures exploratory behavior, we compared the exploratory scores from the maze with the behavior of ants in an open field (Fig. 1B,C). We collected 90 more *L. humile* workers on February 2015 from a colony on the UCSD campus and housed them in individual containers. On the first day after collection, each ant was tested in the eight-armed maze. On the second day, each individual was placed in an open plastic box (11×11 cm) with fluon coating its walls to prevent the ant from escaping. We recorded the movement of the ant with a video camera for 5 min. We then used the tracking software AnTracks (https://sites.google.com/view/antracks) to determine the length of each ant's walking path during these 5 min and its path tortuosity, measured as the standard deviation of the turning angle (Adler and Gordon, 1992). We then examined the relationship between the length of travel paths or path tortuosity with the number of spices visited in the eight-armed maze using Pearson's correlation.
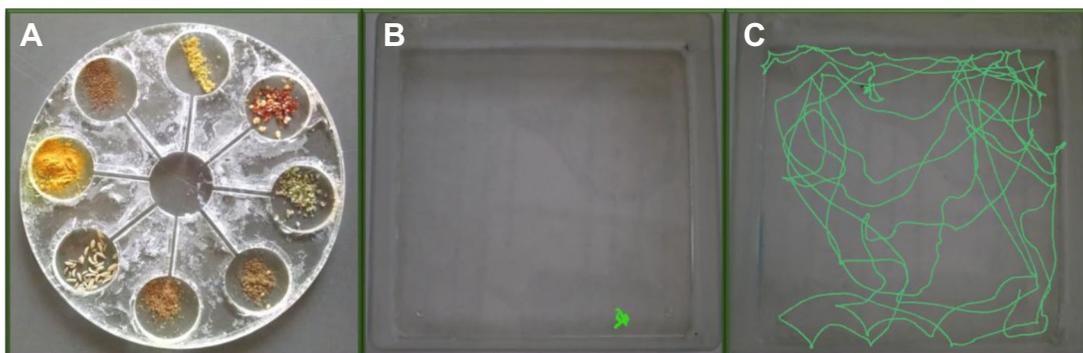
Fig. 1. Quantifying exploration. (A) The eight-armed maze used for quantifying exploration. (B,C) Walking trajectories (green lines) of Argentine ants (*Linephithema humile*) walking in an open field for 5 min. These ants made 0 (B) and 5 (C) visits to spices when tested in the eight-armed maze the previous day.

Gene expression

To determine whether gene expression varies with exploratory behavior, we examined the expression of *Lhfor* in 17 individuals collected in January 2015 from a colony on the UCSD campus. The exploratory behavior of each individual was measured three times using the eight-armed maze, on three consecutive days to obtain an average exploratory behavior. Immediately after testing for exploratory behavior on the third day, during the early afternoon hours, ants were flash-frozen using liquid nitrogen and their heads were separated from their bodies on dry ice and under a microscope with a pair of pre-chilled forceps. Because of the small size of *L. humile* ants, we could not extract brains from the heads and instead we used entire heads, which include other organs aside from the brain, as done in other species of small ants (Warner et al., 2017).

To extract RNA for qPCR analysis, individual heads were placed in a 1.5 ml microcentrifuge tube and homogenized with a glass pestle in 0.5 ml of TRIzol® Reagent (Thermo Fisher Scientific cat. no. 15596-018). Total RNA was extracted using Zymo Research Direct-zol RNA MiniPrep (Zymo cat. no. R2053) followed by DNAseI treatment on column following the manufacturer's instructions. Extracted RNA was dissolved in RNase-free water and stored at −80°C. The RNA concentration and purity of each sample (A260/280∼1.9) was measured using a NanoDrop ND-1000 (Thermo Fisher Scientific). All RNA was assessed for integrity using a TapeStation (Agilent Technologies), and the 17 samples with RNA integrity number (RIN) >8.0 were used. A total of 20 ng of total RNA was transcribed with qScript™ XLT cDNA SuperMix (Quanta Bioscience). cDNA was stored at −80°C and briefly transported on dry ice before being thawed and used for qPCR assay. Primer sequences (F: ACTCCTTCCGGATCATCAGC, R: CAAGCAACCTGACACAACGG) for amplifying 129 bp were designed to target the 4th and 5th exons of the *Lhfor* gene (LOC105678094, XM_012377134.1) A species-specific 110 bp fragment of glyceraldehyde-3-phosphate dehydrogenase 1 (LOC105675989) mRNA was used as a reference gene (F: CGA-TTCCATGGGCAAAAGCC, R: AATGACTTTCTTCGCACCG-C). RT-qPCR with 2 ng input cDNA per reaction in triplicate was performed with a BioRad CFX384 Real Time system in 10 µl reaction volume using PerfeCTa® SYBR® Green FastMix® (cat. no. 95072-250) followed by melting curve analysis to ensure homogeneity of the reaction product. RT-qPCR data were analyzed using BioRad CFX manager software. Differences in *Lhfor* mRNA levels were calculated using the comparative ΔC_T method (Schmittgen and Livak, 2008). All expression data of *Lhfor* were normalized to the housekeeping gene *GAPDH* (see primers above), which exhibited low variability across samples (Thellin et al., 2009). We examined the relationship between exploratory behavior and the normalized expression of *Lhfor* using Pearson's correlation.

Allocation of exploratory individuals in nature

To examine where exploratory individuals are found in natural colonies we collected *L. humile* workers from four colonies on the University of California, Los Angeles (UCLA) campus in 2017 (exact dates below) at three different locations per colony: (1) within the nest, (2) from low-use trails and (3) from high-use trails. Trail usage was determined by the density of ants on the trails approximately 3 m from the nest entrance. To determine ant density, we counted the number of ants that passed one point on the trail for 30 s. Each trail's density was measured three times, once every 5 min (Fig. 2). Trail usage was compared with a one-sided, paired Wilcoxon signed-rank test for each time of year (see time of
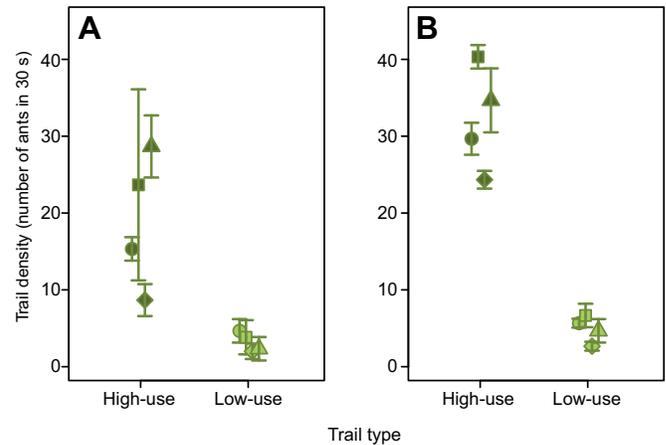
Fig. 2. Quantifying trail use. Trail density, measured as number of ants crossing an invisible line on the trail during 30 s. Values are averages of three counts on each high- and low-use trail, at the end of winter (A) and in early-mid spring (B) in four colonies. Points are averages of three counts and error bars are the standard deviation of these counts. Each colony is denoted by a different shape. Points and error bars are slightly jittered along the *x*-axis to improve visibility.

year definitions below). The four colonies sampled were at least 100 m apart to ensure independent samples from functionally distinct colonies (Heller et al., 2008).

Approximately 30 individuals were collected first from the high-use trail, then from the low-use trail (approximately 10 m from the nest entrance on each type of trail) and finally from inside the nest (90 ants total per colony). This order of collection was used to avoid any disturbance that the collection of ants from the nest might have had on trail usage. Ants were brought back to the laboratory the same day they were collected, and each individual was stored in an individually labeled cup (with water and sugar water provided *ad libitum*) to ensure individual identification in repeated testing. The exploratory behavior of each ant was examined using the eight-armed maze twice over 2 days – once on each day. Because some ants died between the two days, at least 15 individuals were tested twice from each location. After the second trial was completed, all ants were released back to their nest.

To test for differences in exploratory behavior throughout the year and in the allocation of exploratory individuals to various locations, we conducted the above procedure twice: first at the end of winter (3 February–10 March 2017), when the availability of natural resources is low, and then in early-mid spring (18 April–18 May 2017), when the availability of natural resources is increasing. The average temperature at the end of winter (14°C) was 3°C colder than in early-mid spring (17°C), and the air was drier at the end of winter (dew point 8.24°C) compared with early-mid spring (dew point 10.08°C). Weather data were obtained from historical records from the closest weather station to UCLA, at Santa Monica (KSMO), through 'Weather Underground' (https://www.wunderground.com/history). Ants from the four colonies were collected in the same order during both times of year to ensure that the number of days between the two collection periods was approximately equal for each colony.

Differences in exploratory behavior between times of year and collection locations were examined using a generalized linear mixed model (GLMM) with a log-link function using the lme4 R package (Bates et al., 2015). The dependent variable was the number of visits ants made to spices in the eight-armed maze during 5 min. The fixed

effects were collection location (nest, low-use and high-use trails) and time of year (end of winter and early-mid spring) and the random effects were colony identity and the day on which the exploratory behavior of an ant was tested (first or second; each ant was tested twice). To determine the confidence of our estimates, we ran a Wald chi-squared test using the Anova R function 'MASS' package (Venables et al., 2002) on the GLMM results. *Post hoc* comparisons between collection sites for each time of year were conducted with Tukey tests using the 'lsmeans' R package (Lenth, 2016). All analysis was conducted in R version 3.1.2 (https://www.r-project.org/).

RESULTS

Exploratory behavior

Exploratory behavior is a repeatable trait. The 66 ants whose exploratory behavior was examined over three consecutive days had a repeatability score of 0.28 (CI: 0.13, 0.44) for the number of visits that ants made to spices, measured with ICC. This repeatability score is comparable to that found in other animals (Bell et al., 2009).

Exploratory behavior quantified with the eight-armed maze reflected the ants' movement in an open field. Path length significantly and positively correlated with the number of visits an ant made to spices in the eight-armed maze in the preceding day (Pearson's correlation: $r=0.28$, $P=0.01$; Fig. 3). Interestingly, we did not detect a significant relationship between an ant's path length and its tortuosity (Pearson's correlation: $r=-0.16$, $P=0.16$), nor was there a significant relationship between path tortuosity and the number of visits to spices in the eight-armed maze (Pearson's correlation: $r=0.03$, $P=0.77$).

Gene expression

Exploratory behavior is related to the expression of *Lhfor*. We found a significant negative correlation between the number of visits an ant made to spices in the eight-armed maze and the expression of *Lhfor* (Pearson's correlation $r=-0.6$, $P=0.01$; Fig. 4).
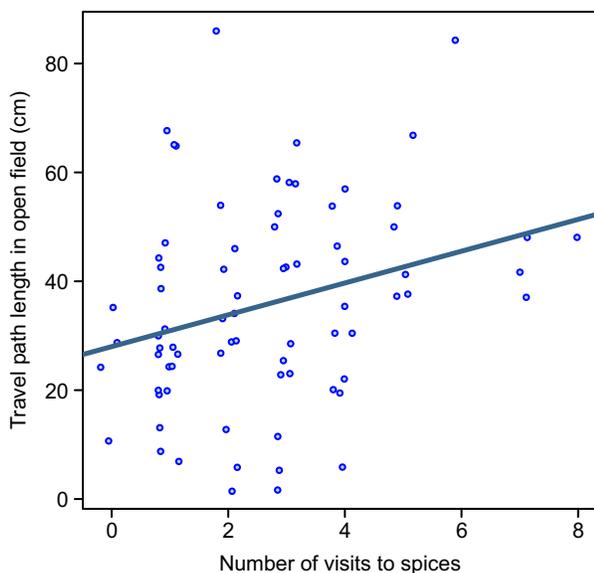
Fig. 3. Exploratory behavior across settings. A significant positive correlation between the exploratory behavior of an ant when measured in the eight-armed maze and the travel path length in an open field. Points are slightly jittered along the *x*-axis to improve visibility.
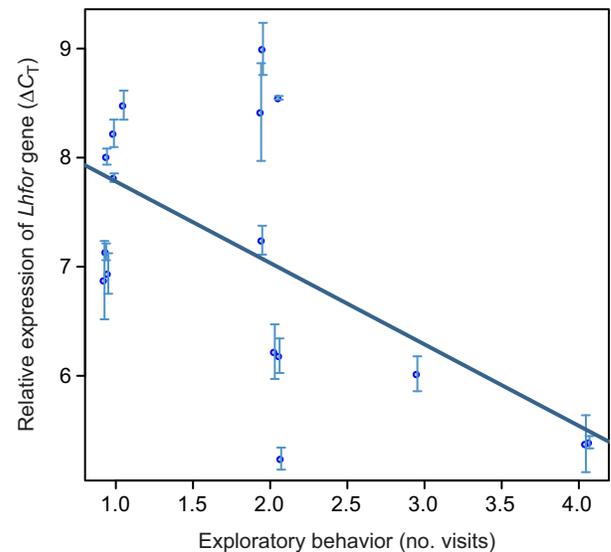
Fig. 4. Exploratory behavior related to gene expression. A significant negative correlation between exploratory behavior (number of visits to spices in the eight-armed maze) and the expression of the *Lhfor* gene (relative gene expression normalized to *GAPDH*). Error bars are standard errors. Points are slightly jittered along the *x*-axis to improve visibility.

Allocation of exploratory individuals in nature

Linepithema humile colonies allocate exploratory individuals to where they are most needed. High-use foraging trails were statistically significantly denser than low-use trails in both times of year (one-sided, paired Wilcoxon test: end of winter: $V=120$, $P=0.0003$; early-mid spring: $V=78$, $P=0.001$; Fig. 2). At both times of year, the most exploratory individuals were found on the low-use trails and the least exploratory individuals were found inside the nest. Individuals on the high-use foraging trail exhibited intermediate levels of exploratory behavior at the end of winter and exploratory behavior similar to workers from inside the nest in the early-mid spring (GLMM location effect: $\chi^2=53.64$, d.f.=2, $P<0.0001$; Fig. 5). Ants were significantly more exploratory in the early-mid spring than at the end of winter (GLMM time of year effect: $\chi^2=12.43$, d.f.=1, $P<0.0005$; Fig. 5). The random effects had very little impact on the model (variance for 'colony'=0.01, and for 'day on which the ant was tested'=0.02).

DISCUSSION

The amount of exploration an *L. humile* worker exhibits is a repeatable trait that is correlated with the expression of *Lhfor*. In addition to identifying the relationship between gene expression and exploratory behavior of individual workers, we showed that colonies allocate exploratory individuals to where they are most needed ecologically. Thus, our work links individual and collective behaviors mechanistically and functionally from the level of the genes through individuals to the society.

Many behavioral traits are repeatable; however, the mechanisms that underlie such repeatability are understudied. The exploratory behavior of *L. humile* was found to be as repeatable as some behaviors studied in other animals (Bell et al., 2009). Thus, on a time scale of a few days, individuals maintain a certain level of exploratory behavior. Further work is required to determine whether exploration changes on longer time scales, for example, as individuals age or when they switch their task, such as from nurse to forager.
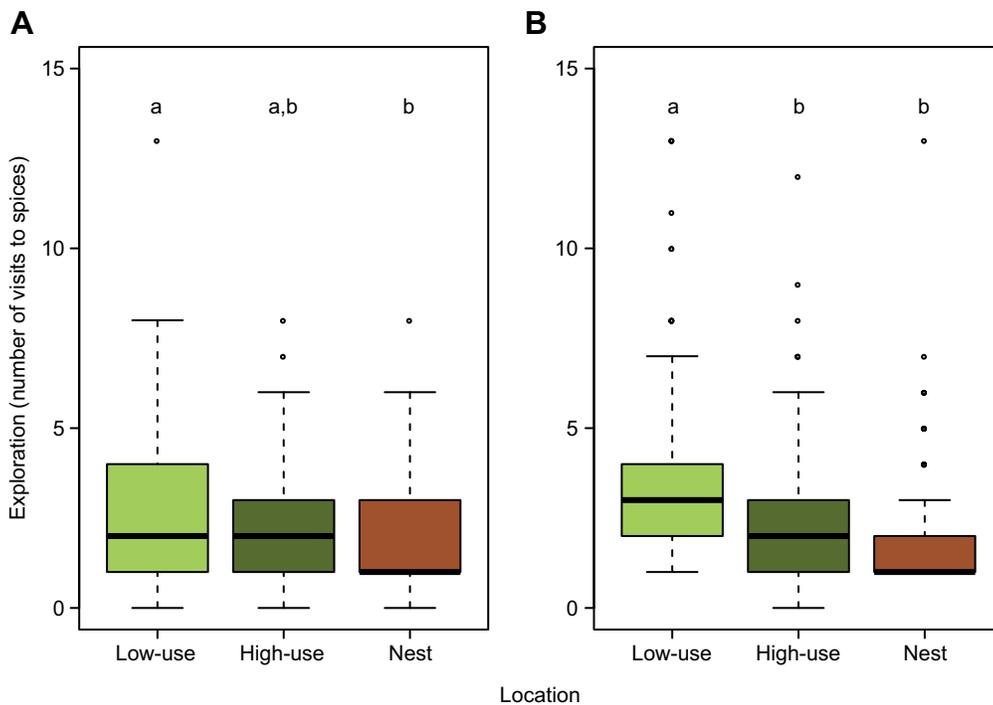
Fig. 5. Exploratory behavior in nature. Exploratory behavior of *L. humile* workers collected from different locations: low- and high-use foraging trails, and from inside the nest in the (A) late winter and (B) early-mid spring. Boxes indicate the lower and upper quartiles; horizontal lines within boxes indicate the median; whiskers extend to the 1.5 interquartile range from the box; and open circles indicate outliers. Boxes that do not share a letter are statistically different according to a *post hoc* Tukey test (*P*<0.01).

We identified that the expression of *Lhfor* decreases with exploratory behavior. The effect of gene expression on a behavior allows for behavioral persistence on certain time scales and flexibility on other time scales. For example, exploratory behavior may persist as long as the appropriate level of expression of *Lhfor* is maintained, and when this expression changes, the behavior may change as well. Foragers of harvester ants have the lowest expression of *Pbfor* in the colony compared with workers performing other tasks (Ingram et al., 2005). Foragers spend more time than any other workers outside the nest because they travel to retrieve food. Thus, the low expression of *Lhfor* in exploratory *L. humile*, who take longer travel paths than other workers, is consistent with its low expression found in foragers of harvester ants. Interestingly, an opposite trend is found in the red imported fire ant, *Solenopsis invicta*. In that system, *Sifor* is over-expressed in foragers and colonies with highly exploratory individuals express more *Sifor* (Bockoven et al., 2017). In another insect, *D. melanogaster*, 'rovers' larvae that explore their environment while foraging have higher expression of the gene *foraging* than 'sitters' that do little exploration during foraging (Osborne et al., 1997). Thus, species differ in the way that gene expression relates to behavior. A recent study (Ingram et al., 2016) found both daily oscillations in the expression of the foraging gene as well as differences among tasks. Changes that occur in gene expression at the temporal scale of hours are influenced by the amount of light that foragers (but not nurses) are exposed to (Ingram et al., 2016). Thus, it is possible that the exposure of exploratory individuals to light when they are outside the nest may control the expression of *Lhfor*. This hypothesis may be tested by comparing the expression of *Lhfor* between exploratory individuals from low-use trails and less exploratory individuals from high-use trails. Both of these workers potentially have a similar exposure to light but they differ in their exploration. Finally, the *foraging* gene has been linked with memory and learning in *D. melanogaster* larvae (Kaun et al., 2007). Therefore, it is possible that the relationship between the expression of this gene and exploratory behavior in *L. humile* may relate to links between exploratory behavior and learning (Cook et al., 2018).

Exploratory behavior has ecological significance at both the individual and collective levels. First, we showed that exploratory behavior, quantified in a simple eight-armed maze, is equivalent to the more ecologically relevant travel path length in an open field (Fig. 3). Interestingly, path tortuosity did not relate to travel path length in an open field or to the exploration of an eight-armed maze. Previous work showed that as the path tortuosity of ants' paths increases, interaction rate decreases (Pinter-Wollman et al., 2011). Thus, our inability to detect a relationship between path tortuosity and exploratory behavior suggests that exploratory behavior does not necessarily affect interaction patterns, which are known to regulate collective actions, such as foraging activity (Greene and Gordon, 2007; Pinter-Wollman et al., 2013). Thus, the amount of exploratory behavior exhibited by ants in a colony likely has a greater impact on the area a colony can reach, rather than on how the colony coordinates its activities, because exploratory ants travel far.

Exploratory individuals were found where we expected them. In natural conditions, we expect ants that have high exploratory behavior to cover more ground than other individuals. More exploratory individuals might be more likely to find new food sources, and low-use trails are likely new trails that have not been established and have not yet become high-use trails. Indeed, we found that ants on low-use trails were more exploratory than those on high-use trails. Half of the low-use trails went into trees (H.P., personal observations), where ants often feed on honeydew from aphids. Aphids can move around, and so it is possible that exploratory behavior would help locate them. In contrast, high-use trails went towards trash cans (H.P., personal observations), which are reliable, constant food sources that do not move, and therefore do not require much exploration. Previous work on high-use trails (Flanegan et al., 2013) showed that certain individuals will occasionally leave these trails and find new resources that are adjacent to the trail. It is possible that these meandering ants are more exploratory than individuals on the main trail and that variation in exploratory behavior on high-use trails is beneficial for finding new resources along the path to an established resource.

Tasks performed inside the nest, such as nursing and cleaning, likely do not require as much exploratory behavior as tasks performed outside the nest, such as seeking for new food sources, because the space inside the nest is smaller than that outside. Indeed, ants outside the nest, especially on low-use trails, were significantly more exploratory than ants inside the nest. It is possible that the similarity between exploratory behavior on high-use trails and inside the nest, especially in early-mid spring (Fig. 5), is a result of the way we sampled ants from inside the nest. If many foragers are waiting near the nest entrance to be recruited, sampling ants from the top portion of the nest, as we did here, would potentially include a greater proportion of foragers than samples from deeper in the nest, which may reach more brood caretakers. Thus, sampling ants from deeper inside the nest might have produced a greater difference in exploratory behavior between ants collected in the nest and on high-use trails; however, such sampling would be destructive and might have disturbed colony activity.

Finally, exploratory behavior was more predominant in the early-mid spring, when more food is available and when *L. humile* colonies expand their local range (Heller and Gordon, 2006), compared with at the end of winter. Thus, colonies allocate exploratory individuals both spatially and temporally, based on the collective 'needs' of the colony. It is possible that when the weather is warmer, ants move faster and therefore are more exploratory. It is further possible that temperature plays a role in the expression of *Lhfor*, just as exposure to light does (Ingram et al., 2016). Further studies of the proximate mechanisms that underlie differences in behavior at the individual will uncover the ways in which the emergent collective behavior of the colony responds to changes in its environment.

By linking individual and collective behaviors, using both proximate and ultimate perspectives, our work brings us closer to uncovering how collective behaviors emerge. The genetic, mechanistic control of variation in behavior among individuals acts in conjunction with the functional, adaptive requirements of the colony for workers that express different behaviors in different locations. Further work on the feedback between how environmental pressures affect the genetic mechanisms and how the genetic mechanisms affect behavioral responses to environmental changes are necessary to elucidate the evolution of behavior.

Competing interests

Author contributions
Conceptualization: N.P.-W.; Methodology: N.P.-W., H.P., A.S., A.P.; Validation: N.P.-W.; Formal analysis: N.P.-W., H.P., A.P.; Investigation: N.P.-W.; Resources: N.P.-W.; Data curation: N.P.-W.; Writing - original draft: N.P.-W.; Writing - review & editing: N.P.-W., H.P., A.P., A.S.; Visualization: N.P.-W.; Supervision: N.P.-W.; Project administration: N.P.-W.; Funding acquisition: N.P.-W.

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Data availability
Data are available from the Dryad Digital Repository (Page et al., 2018): dryad.ft80s9n

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