Ant collective behavior is heritable and shaped by selection

Running title: The evolution of collective behavior

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Abstract

Collective behaviors are widespread in nature are usually assumed to be strongly shaped by natural selection. However, the degree to which variation in collective behaviors is heritable and has fitness consequences -- the two prerequisites for evolution by natural selection -- is largely unknown. We used a new pharaoh ant (*Monomorium pharaonis*) mapping population to estimate the heritability, genetic correlations, and fitness consequences of three collective behaviors (foraging, aggression, and exploration) as well as body size, sex ratio, and caste ratio. Heritability estimates for the collective behaviors were moderate, ranging from 0.22 to 0.40, but lower than our estimates for the heritability of caste ratio, sex ratio, and the body size of new workers, queens, and males. Moreover, the collective behaviors were phenotypically correlated and in some cases genetically correlated, suggesting that they form a suite of correlated traits. Finally, we found evidence for directional, stabilizing, and disruptive selection that was similar in strength to estimates of selection in natural populations. Disruptive selection was very common and may act to maintain behavioral variation. Altogether, our study begins to elucidate the genetic architecture of collective behavior and is one of the first studies to demonstrate that it is shaped by selection.

Keywords: Collective behavior, animal personality, genetic correlation, heritability, selection, caste ratio

Introduction

Collective behavior is ubiquitous in nature. Examples include predator avoidance in schools of fish, the migration of flocks of birds, and nest building in social insects. Researchers are increasingly interested in documenting patterns of variation in collective behavior between groups (i.e. describing collective or group personality; Jandt et al. 2014; Bengston & Jandt 2014; Wright et al. 2019) with a goal of understanding the evolutionary causes and consequences of variation in collective behavior. However, the degree to which collective behaviors are heritable and how genetic variation contributes to population-level variation in individual and collective behaviors remains largely unknown. Furthermore, it is often assumed that, like individual behavior and other individual-level traits, collective behavior and other group-level traits are strongly shaped by natural selection (Gordon 2013, 2016). However, very little is actually known about how natural selection acts on collective behaviors or group-level traits more generally (Wright et al. 2019).

Given that trait variation must be heritable in order for the trait to respond to selection and evolve over time, quantifying heritability is the crucial first step in studying trait evolution (Falconer & Mackay 1996; Lynch & Walsh 1998). Previous studies in ants, honey bees, and sticklebacks suggest that collective behaviors and other group-level traits are heritable (Linksvayer 2006; Hunt et al. 2007; Wark et al. 2011; Greenwood et al. 2015; Friedman & Gordon 2016). Additionally, candidate gene studies have linked allelic variation to variation in collective behavior, providing further evidence that collective behavior is heritable (Krieger 2005; Wang et al. 2008; Wang et al. 2013; Tang et al. 2018). Although numerous studies have examined the genetic architecture of group-level traits in honey bees (Rinderer et al. 1983; Collins et al. 1984; Milne 1985; Moritz et al. 1987; Bienefeld & Pirchner 1990; Pirchner &

Bienefeld 1991; Harris & Harbo 1999; Boecking et al. 2000; Hunt et al. 2007), we know little about the genetic architecture or the evolution of collective behavior and other group-level traits in other group-living species.

Another key factor affecting the relationship between genotype, phenotype, and selection is the pattern of genetic correlations, i.e. the proportion of variance that two traits share due to genetic causes. Genetic correlations can either accelerate or slow down the rate of evolutionary response to selection, depending on the direction of the correlation relative to the direction of selection on the traits (Lynch & Walsh 1998; Wilson et al. 2010). Understanding genetic correlations is especially important for the study of behavioral evolution since behaviors are often thought to be correlated with each other, forming sets of tightly linked traits that are often described as behavioral syndromes (Sih et al. 2004; Dochtermann & Dingemanse 2013). Although genetic correlations have been estimated for individual-level behaviors (reviewed by van Oers et al. 2005), few studies have examined genetic correlations between collective behaviors or other group-level traits, except for in honey bees (Milne 1985; Bienefeld & Pirchner 1990; Boecking et al. 2000).

To fully understand a trait's potential evolutionary response to selection, we must also understand how natural selection acts on it. Knowledge of the fitness consequences of trait variation allows researchers to characterize the type (e.g., directional, stabilizing, or disruptive) and strength of natural selection acting on a trait (Lande & Arnold 1983; Arnold & Wade 1984; Janzen & Stern 1998; Morrissey & Sakrejda 2013). Many studies have estimated the fitness consequences of individual-level behavioral variation (reviewed by Smith & Blumstein 2008), but the consequences of group-level variation have received relatively little attention (but see Wray et al. 2011; Modlmeier et al. 2012; Blight et al. 2016a; Blight et al. 2016b).

Social insects are well-established models for studying collective behavior. Well-studied collective behaviors include nest choice in acorn ants (*Temnothorax* spp.; Möglich 1978; Franks et al. 2003; Pratt 2017), nest defense and hygienic behavior in honey bees (*Apis mellifera*; Spivak 1996; Breed et al. 2004; Evans & Spivak 2010), and pheromonal regulation of foraging in pharaoh ants (*Monomorium pharaonis*; Beekman et al. 2001; Sumpter & Beekman 2003; Robinson et al. 2005). The collective behavior of colony members also shapes colony productivity and the relative investment in workers versus reproductives (i.e. caste ratio) and reproductive males versus queens (i.e. sex ratio). Social insect sex ratio and caste ratio have long served as important models for empirically testing predictions from inclusive fitness theory regarding predicted conflicts between queens and workers over sex ratio and caste ratio (Trivers & Hare 1976; Reuter & Keller 2001; Mehdiabadi et al. 2003; Bourke 2015). However, despite this long-term intense interest in the evolution of colony-level traits, we still know empirically very little about the key parameters governing the evolution of these traits. Indeed, while recent molecular studies have begun to characterize the genomic, transcriptomic, and epigenetic differences between species, between castes within a species, and between individual workers (Friedman & Gordon 2016; Gospocic et al. 2017; Warner et al. 2017; Chandra et al. 2018; Walsh et al. 2018), little is known about the genetic architecture of collective behavior, caste ratio, and sex ratio (Linksvayer 2006). Similarly, while it is clear that colony-level phenotypes can be shaped by patterns of selection within- and between-colonies (Owen 1986; Moritz 1989; Ratnieks & Reeve 1992; Tsuji 1994, 1995; Banschbach & Herbers 1996; Tarpy et al. 2004), few studies have attempted to empirically quantify patterns of selection acting on social insect traits.

In this study we used a genetically variable laboratory population of pharaoh ants (*Monomorium pharaonis*) that we created by systematically intercrossing eight initial parental

lineages throughout the past 12 years. Such a mapping population has proven powerful to elucidate the genetic architecture of a range of traits, including behavioral traits, in mice, rats, and fruit flies (Hansen & Spuhler 1984; Mott et al. 2000; Valdar et al. 2006; King et al. 2012). We first assayed colony-level foraging, aggression, and three measures of exploration using three replicate sub-colonies of 81 distinct colony genotypes of known pedigree (243 replicate sub-colonies total). We chose these three behaviors because they are linked to colony success in other social insects, including ants (Wray et al. 2011; Modlmeier et al. 2012; Blight et al. 2016; Blight et al. 2016). Furthermore, we measured colony productivity, caste and sex ratio, and worker, gyne, and male body size. Next, we used the pedigree information of the colonies to estimate the heritability of and genetic correlations between all traits. Finally, we estimated the strength and pattern of selection acting on all the measured phenotypes in the laboratory.

Materials and Methods

(a) Background and overall design

All *M. pharaonis* colonies used in this study were reared in the lab and derived from eight initial lab stock colonies that have been systematically interbred since 2008 (Pontieri et al. 2017). We maintained all colonies at 27 ± 1 °C and 50% relative humidity on a 12:12 hour light:dark cycle. We split colonies into three equally-sized replicates. All replicate colonies initially consisted of 4 queens, 400 ± 40 workers, 60 ± 6 eggs, 50 ± 5 first instar larvae, 20 ± 2 second instar larvae, 70 ± 7 third instar larvae, 20 ± 2 prepupae, and 60 ± 6 worker pupae. These numbers represent a typical distribution of developmental stages in a relatively small *M. pharaonis* colony (Warner et al. 2018). Except when starving the replicate colonies (see below), we fed all replicate colonies twice per week with an agar-based synthetic diet (Dussutour &

Simpson 2008) and dried mealworms. The replicate colonies always had access to water via water tubes plugged with cotton. Colonies nested between two glass slides (5 cm x 10 cm). We kept all colonies in a plastic colony container (18.5 cm x 10.5 cm x 10.5 cm) lined with fluon and surrounded by a moat of oil to prevent the workers from escaping the box.

After setting up the replicate colonies, we gave the colonies two weeks to acclimate to the new conditions before conducting behavioral assays. We fed the replicate colonies during the first week and removed all food from the colony container at the start of the second week. We starved the replicate colonies for a week so that they would be motivated to forage for food the following week. We conducted the exploratory and foraging assays during the third week and the aggression assays during the fourth week. We fed the replicate colonies after the foraging and aggression assays and then again twice per week starting in the fifth week.

(b) Behavioral observations

(i) Exploratory assay

We assayed the exploratory behavior of both entire colonies and groups of five foragers. We conducted the assay inside a filming box with LED lights arranged along the walls and a camera mounted on the top to film the arena from above (**Supplemental figure 1**). We covered the floor of the box with poster board that we replaced between each assay to remove trail pheromones. We first collected five foragers, defined as any worker outside the nest, from inside the colony container and placed them in a large petri dish. We placed the petri dish upside-down in the middle of a circular arena in the center of the filming box. We waited five minutes to give the workers time to settle down after being handled. After the five minutes, we gently removed the petri dish so the workers were free to move around the arena and filmed the workers exploring the arena for 15 minutes.

Next, we replaced the poster board inside the filming box and placed the five foragers, all remaining foragers from inside the colony container, and the nest containing the rest of the workers, the queens, and the brood inside a petri dish. We placed the petri dish containing the entire colony upside-down in the center of the arena and waited five minutes before lifting the petri dish and filming for 15 minutes.

We analyzed the videos of the five foragers using custom made tracking software (https://github.com/swarm-lab/trackR) to track the location of each ant in each frame of the video. We wanted to only include tracking information when the trajectory of the ants was not influenced by the wall of the arena so we removed all tracks where the ants were within 3 mm of the wall, resulting in many separate trajectories within each video for each ant. Next, for each sub-trajectory, we calculated the net squared displacement (NSD) by taking the square of the distance traveled by each ant between the starting location and each successive location along the rest of the trajectory. To calculate the diffusion coefficient, we took the slope of linear portion of the plot of NSD over time and fit the equation:

$$MSD = 4Dt$$

where mean squared displacement (*MSD*) is the slope of NSD over time, *D* is the diffusion coefficient, and *t* is time (Börger & Fryxell 2012). The diffusion coefficient served as a measure of how quickly the ants dispersed from their nest.

In addition, for both the five forager and entire colony videos, we calculated the arena coverage and coverage redundancy over time. First, we computed the absolute difference between each frame of the recorded video and a background image of the experimental setup without ants in it. When a pixel had a large absolute difference, it meant an ant was present on that pixel in a given frame. We then applied a threshold to the difference image and classified all

the pixels with a difference value above the threshold as "ant-covered" pixels and gave them a value of 1, and all the pixels with a difference value below the threshold as "background" pixels and gave them a value of 0. Finally, we computed the cumulative sum of the segmented images over time and calculated for each of them the arena coverage as the percentage of the pixels with a value of at least 1 (i.e. what fraction of pixels have been visited by ants at least once), and the coverage redundancy as the average value of the pixels with a value of at least 1 (i.e. of the visited pixels, how many times in average have they been visited by ants) (**Figure 1, Supplemental figure 2**).

We will refer to three exploratory behaviors as "exploratory rate", "group exploration", and "colony exploration". "Exploratory rate", refers to the diffusion coefficient, "group exploration" to the percent of the arena covered by the groups of five foragers, and "colony exploration" to the percent of the arena covered by the entire colony.

(ii) Foraging assay

We conducted the foraging assay on each replicate colony the day after the exploratory assay. We melted the agar-based synthetic diet and soaked a cotton ball in the liquid. When the cotton ball solidified, we placed it on the plateau of a 3D printed ramp (**Supplemental figure 3**). We placed a colony container inside the filming box and put the ramp against the wall on the opposite side of the nest. Once an ant first discovered the food, we started filming and filmed for one hour. If no ant discovered the food in 30 minutes, we started the recording. We calculated the foraging rate by manually counting the number of ants that reached the plateau of the ramp in each video. Because many ants went back and forth from the food to the nest, we counted many ants more than once.

(iii) Aggression assay

Like other unicolonial ant species, *M. pharaonis* workers show little to no aggression towards *M. pharaonis* workers from other colonies (Schmidt et al. 2010). To get *M. pharaonis* workers to act aggressively, and to be able to quantify aggression against a constant "enemy" for all of our experimental colonies, we used workers from a single *Monomorium dichroum* colony that had been kept in the lab under the same conditions as the *M. pharaonis* colonies for 5 years. We conducted the aggression assays a week after the foraging assays. We first collected twenty foragers of both species and placed them in separate small petri dishes (**Supplemental figure 4**). We placed both small petri dishes upside down in a large petri dish for five minutes before lifting both petri dishes and allowing the workers of both species to interact. Every 5 minutes for one hour, we recorded the number of *M. pharaonis* workers that were biting *M. dichroum* workers. We defined aggression as the average number of *M. pharaonis* workers biting *M. dichroum* workers across all observations within an hour. We froze all of the ants used in the aggression assay so that we did not reuse *M. dichroum* workers in more than one assay.

(c) Colony productivity and body mass measurements

As a measure of colony productivity, we surveyed each replicate colony once per week and counted the number of workers and brood at all developmental stages. *M. pharonis* colonies usually only produce new gynes (virgin queens) and males in the absence of fertile queens (Edwards 1991; Warner et al. 2018). Therefore, in order to induce the production of new gynes and males, we removed queens at the start of the fifth week, after the aggression assay. We conducted weekly surveys until all brood matured into worker, gyne, or male pupae. In addition to colony productivity data for the total number of workers, gynes, and males produced, the weekly surveys also allowed us to calculate colony caste and sex ratio. We defined caste ratio as the number of gynes relative to the total number of females produced, and sex ratio as the

number of gynes relative to the total number of reproductives (gynes and males) produced. To measure body size, we collected 15 worker pupae,10 gyne pupae, and 10 male pupae from each replicate colony. We dried the pupae out in a drying oven for 24 hours before weighing.

(d) Statistical Analyses

We performed all statistical analyses in R version 3.4.1 (R Core Team 2014). We estimated the repeatability of all measured phenotypes across replicate colonies using a generalized linear mixed model (GLMM) approach in the R package MCMCglmm (Hadfield 2010). We used linear models to evaluate whether block, queen age, and *Wolbachia* infection status (two of the original eight lineages included in the heterogeneous stock were infected with *Wolbachia* (Schmidt et al. 2010, Pontieri et al. 2017) had an effect on any of the phenotypes. If so, we include them as fixed effects in the GLMM. We included colony identity as a random effect.

We utilized the known pedigree of our *M. pharaonis* colonies to estimate the heritability of, and genetic correlations between, all measured phenotypes. We used the R package MCMCglmm to run animal models using a Bayesian Markov chain Monte Carlo (MCMC) approach (de Villemereuil 2012). Animal models estimate genetic parameters of a trait by evaluating the phenotypic covariance between all pairs of individual "animals" (in our case, individual colony genotypes) in a pedigree (Kruuk 2004). We accounted for the fact that ants are haplodiploid (males are haploid, females are diploid) by constructing the pedigree as if the traits were all sex-linked. We used weakly informative priors for 1,000,000 iterations, with a burn-in period of 10,000 iterations and stored estimates every 500 iterations (full R script included in supplemental material; following de Villemereuil 2012). We assessed convergence of the models by visually inspecting estimate plots and assessing the autocorrelation values (de Villemereuil

2012). We used linear models to evaluate whether block, queen age, and *Wolbachia* infection status (Singh et al. 2019) had an effect on any of the phenotypes. If so, we included them in the animal models. We analyzed whether behaviors were phenotypically correlated with each other (i.e. behavioral syndromes) using Spearman rank correlations. We corrected for multiple comparisons by using the "FDR" method in the R function "p.adjust."

We estimated the strength of selection using a standardized selection gradient approach as described by Morrissey & Sakrejda (2013). This method is similar to the approach outlined by Lande and Arnold (1983) and uses spline-based generalized additive models to model the relationship between fitness and traits. All behaviors were normalized to a mean of 0 and a standard deviation of 1 so that the selection estimates represent standardized values. We included *Wolbachia* infection status (Singh et al. 2019), queen age, and block number in all models. We estimated selection gradients and prediction intervals after 1000 bootstrap replicates (Morrissey & Sakrejda 2013). In order to elucidate how selection acts simultaneously on two traits, we produced three dimensional plots for fitness over each combination of the five behavioral variables.

Results

(a) Repeatability and heritability estimates

All five behaviors, caste and sex ratio, and worker, gyne, and male body mass were significantly repeatable across replicate colonies (**Supplemental table 1**). We estimated the heritability of the five collective behaviors to be between 0.22 and 0.40 (**Figure 2)**. We estimated the heritability of worker body mass to be 0.38, gyne body mass to be 0.57, and male body mass to be 0.53 (**Figure 2)**. Finally, we estimated the heritability of caste and sex ratio and five colony

productivity measures to be between 0.14 and 0.75 (**Figure 2)**. The 95% confidence intervals of the heritability estimates of all traits were above zero.

(b) Phenotypic and genetic correlation estimates

We found phenotypic correlations among the five measured collective behaviors (**Figure 3**). Foraging rate was negatively correlated with aggression and positively correlated with both group exploration and colony exploration. Aggression was negatively correlated with exploratory rate. Group exploration and colony exploration were positively correlated. Although most of the genetic correlation estimates were greater than or less than zero, we found only one correlation, between foraging and colony exploration, with a 95% CI that did not overlap with zero (see **Figure 3** and **Supplemental table 2** for estimates and 95% CI). Additionally, although the 95% CI overlap with zero, the genetic correlations between foraging and aggression and foraging and group exploration were less than -0.20 and greater than 0.20, respectively. The genetic correlation estimates between behaviors and all other traits, as well as among all the other traits, were mostly small and all had 95% CI that overlapped with zero (**Supplemental table 2**).

(d) Selection gradients

When defining fitness as the number of reproductives (gynes + males) produced by the colony, we found evidence for positive linear selection on foraging, and negative linear selection on aggression, exploratory rate, and colony exploration (**Table 1, Figure 4**). Additionally, we found evidence for disruptive (positive quadratic) selection for aggression and exploratory rate and stabilizing (negative quadratic) selection for colony exploration. When defining fitness as the number of workers produced by a colony, we found evidence for positive linear selection for

foraging, group exploration, and colony exploration and negative linear selection for aggression and Exploratory rate (**Table 1**). Finally, we found evidence for disruptive selection for exploratory rate and colony exploration and stabilizing selection for aggression.

When defining fitness as the production of new reproductives, we found evidence for negative selection on worker body mass and positive selection on gyne body mass (**Supplemental table 4**). Additionally, we found evidence for disruptive selection on worker, gyne, and male body mass. When defining fitness as the production of new workers, we found evidence for negative selection on worker body mass and positive selection on gyne and male body mass (**Supplemental table 4**). Finally, we found evidence for disruptive selection on worker, gyne, and male body mass.

To further put our results into context, we estimated the proportion of variance among our colonies for both measures of fitness (the productions of new reproductives and workers) that was explained by variation in any of our five behavioral variables, experimental block, or *Wolbachia* infection status. For the production of new reproductives, we found that aggression explained the largest amount of the variance (5.29%), followed by foraging (2.29%), group exploration (1.94%), exploratory rate (0.52%), and colony exploration (0.33%) (**Supplemental table 5**). For the production of new workers, we found that foraging explained the largest amount of the variance (1.29%), followed by aggression (0.53%), colony exploration (0.34%), group exploration (0.27%), and exploratory rate (0.08%) (**Supplemental table 5**).

Discussion

Collective behavior is ubiquitous in nature and presumed to have strong fitness consequences for group members. Moreover, repeatable variation in collective behavior (often

described as collective or group-level "personality") has been commonly observed (Bengston & Jandt 2014; Planas-Sitjà et al. 2015; Jolles et al. 2017; Wright et al. 2019). However, we still know little about the underlying genetic mechanisms generating variation in collective behavior and how collective behavior is shaped by selection. A major difficulty for elucidating the genetic basis of collective behavior is that, unlike individual-level behavior, collective behavior by definition depends on social interactions among members of the group. As a result, the genetic architecture of collective behavior fundamentally depends on the collective genetic make-up of these individuals (McGlothlin et al. 2010; Linksvayer 2015). Quantifying patterns of selection on group-level traits also has an added level of difficulty because the level of replication is the group (e.g., colony) and not the individual. Here, we begin to elucidate the genetic architecture underlying collective behavior and other group-level traits and to characterize how selection acts on these traits in a laboratory population of the ant *Monomorium pharaonis* that we created for this purpose. We provide evidence that variation in collective behaviors, including foraging, aggression, exploratory rate, group and colony exploration, and other group-level traits measured in the laboratory is heritable, phenotypically and genetically correlated, and shaped by selection.

We estimated the heritability of collective behaviors to be between 0.22 and 0.40, which was generally lower than the heritability estimates for body size (0.38 to 0.58), colony productivity (0.14 to 0.75), and caste (0.42) and sex ratio (0.49) (**Figure 1**). These heritability estimates demonstrate that all of the phenotypes we measured, including collective behaviors, have the ability to respond to selection and evolve over time. Although numerous studies have examined the genetic architecture of group-level traits in honey bees (Rinderer et al. 1983; Collins et al. 1984; Milne 1985; Moritz et al. 1987; Bienefeld & Pirchner 1990; Pirchner & Bienefeld 1991; Harris & Harbo 1999; Boecking et al. 2000; Hunt et al. 2007), this is one of the

first studies to examine the genetic architecture or the evolution of collective behavior and other group-level traits in an ant species.

Although our heritability estimates are somewhat higher than other estimates of heritability across animal taxa (e.g. the heritability of individual-level behaviors was on average 0.14; Dochtermann et al. 2015), heritability estimates can vary widely and all-else-equal are expected to be higher in animals bred in captivity than in nature because environmental conditions in the laboratory are controlled (Simmons & Roff 1994). Furthermore, the heritability estimates for all of our measured group-level phenotypes are likely higher than individual-level behaviors because the heritability of traits influenced by social interactions includes the contribution of heritable components of the social environment (Linksvayer 2006; Bijma et al. 2007a;; Bijma et al. 2007b; Linksvayer et al. 2009; McGlothlin et al. 2010; Bijma 2011). There is ample empirical and theoretical evidence that this form of "hidden heritability" contributes to the heritable variation and also the evolutionary response to selection for social traits (Wade 1976; Moore 1990; Muir 2005; Linksvayer 2006; Bijma et al. 2007b; Bergsma et al. 2008; Wade et al. 2010; Bijma 2011). Because we kept all components of the social environment intact across replicate sub-colonies of each colony genotype (i.e. the workers, queens and brood were all from the same parent colony), our heritability estimates do not partition out the relative contributions of variation in the workers' own genomes from variation in the genomes of other colony members (Linksvayer 2006; Linksvayer et al. 2009).

We found evidence for both phenotypic and genetic correlations between collective behaviors. Suites of phenotypically correlated behaviors are termed "behavioral syndromes" and have been documented throughout the animal kingdom, including in social insects (Sih et al. 2004; Jandt et al. 2014). The behavioral syndrome we found in *M. pharaonis* consisted of a

positive correlation between foraging and exploration, which were both negatively correlated with aggression. Our phenotypic and genetic correlation estimates were generally similar. For example, the four strongest genetic correlation estimates (Foraging - Aggression; Foraging - Forager coverage; Foraging - Colony coverage; Forager coverage - Colony coverage; **Figure 2**) were also four of the five significant phenotypic correlations and were all in the same direction. However, our genetic correlation estimates were generally very weak (i.e. not significantly different than zero) and only one of our genetic correlation estimates was bound away from zero (the correlation between foraging and colony exploration).

Traditionally, behavioral ecologists relied on the assumptions that all behavioral traits were heritable, not strongly genetically correlated, and thus free to evolve independently from other traits in response to patterns of selection on each trait. This approach was termed the "phenotypic gambit" (Grafen 1984). Our results generally support these assumptions as we found moderate estimates of heritability for all five behavioral variables and relatively weak genetic correlation estimates. These results suggest that collective behaviors are free to respond to selection, and that the underlying genetic architecture will not constrain long-term optimization by natural selections (Lynch & Walsh 1998; Wilson et al. 2010).

We calculated the strength and direction of selection acting on collective behavior and found evidence for both positive and negative linear selection as well as widespread disruptive selection (**Figure 4, Table 1**). Although colonies showed a consistent positive relationship between foraging and fitness, the relationship between fitness and the other four behavioral variables was more complex, with colonies showing both high and low values often out-performing intermediate colonies. The absolute value of our estimates of the strength of linear selection were between magnitudes of 0.01 and 0.22 (average of 0.10) and between 0.01 and

0.13 (average of 0.08) when defining fitness as the production of reproductives and workers, respectively. These estimates are similar or slightly smaller than estimates of the strength of linear selection in wild populations (median of 0.16 across 63 studies; Kingsolver et al. 2001). The absolute value of our estimates of quadratic selection were between magnitudes of 0.02 and 0.39 (average of 0.13) and between 0.02 and 0.23 (average of 0.12) when defining fitness as the production of reproductives and workers, respectively. Again, these estimates are similar to the quadratic selection in the wild (median of 0.10, Kingsolver et al. 2001).

Understanding the mechanisms maintaining behavioral variation is an important topic in evolutionary biology and behavioral ecology. Although natural selection generally reduces variation from a population by eliminating less fit phenotypes (Fisher 1930), natural selection can actually maintain variation if extreme phenotypes have higher fitness than intermediate ones (i.e. disruptive selection). Interestingly, we found evidence for significant disruptive selection for four of the five behavioral variables for at least one of the two fitness measurements (**Table 1**), suggesting that disruptive selection acts to maintain variation for collective behavior in *M. pharaonis*. Traditionally, disruptive selection was believed to be very rare because if a population's mean trait value resides in a fitness valley, any perturbation would cause the population to climb toward a fitness peak (Endler 1986). However, a meta-analysis on selection estimates on natural populations found that disruptive selection is as common and as strong as stabilizing selection (Kingsolver et al. 2001). Variation in social insect colonies might also be maintained if there is linear selection in opposite directions on the production of reproductives versus workers, but only if colony worker number influences colony survival or affects the likelihood of colony reproduction through budding, whereby a group of workers accompanies queens to start a new colony (Buczkowski & Bennett 2009). Indeed, we found evidence for both

positive and negative linear selection on colony exploration when defining fitness as the production of workers and reproductives, respectively (**Figure 4; Table 1**). Overall, our results suggest that variation in collective behavior in *M. pharaonis* is likely maintained by a combination of disruptive selection and opposing linear selection for the production of workers and reproductives.

We conducted the current study in a laboratory environment, which enabled us to strictly control the demographic make-up (i.e. queen number, worker number, etc.) and precise environmental conditions experienced by the three replicate colonies for each of our 81 colony genotypes. Such control in particular is valuable given the complexity of social insect colonies (Linksvayer 2006; Kronauer & Libbrecht 2018). However, we also acknowledge the caveat that our choice to conduct our study in a controlled laboratory environment likely had strong effects on both our estimates of heritability and genetic correlations, as well as our estimates of the pattern and magnitude of selection. In particular, it is difficult to know how the fitness consequences of variation in collective behavior that we observed would change in a more natural setting. Because *M. pharaonis* tends to be found in association with humans, both in the tropics in their presumed native range (Wetterer 2010) and in heated buildings in introduced temperate regions, the laboratory conditions of our study might be more similar to the "natural" conditions experienced by our study species than other non-synanthropic species. Although future studies conducted in the field would certainly be valuable, a field study on a similar scale as our study is likely not feasible.

Overall, this study increases our understanding of the genetic architecture of collective behavior and demonstrates that it is strongly shaped by natural selection. Future studies should focus on identifying the mechanisms by which genes function to influence collective behavior

and how variation in these genes affects patterns of variation for collective behavior within populations. Candidate gene approaches have been used successfully to demonstrate the roles of the ant ortholog of the *foraging* gene (*ppfor*; Lucas & Sokolowski 2009) and dopamine (Friedman et al. 2018). In addition to candidate gene approaches, future studies should utilize unbiased approaches such as quantitative trait locus (QTL) mapping in mapping populations (e.g., Hunt et al. 1998; 2007) such as ours, and association mapping in natural populations (e.g., Kocher et al. 2018). Additionally, future research should aim to understand the mechanisms underlying the expression of collective behavior. For example, chemical communication (e.g. cuticular hydrocarbons, pheromones) likely plays a large role in regulating collective behavior in social insects. Finally, future studies should seek to disentangle the contribution of workers' own genomes and the composite sociogenome of their nestmates (including other workers, queens, and brood), by using cross-fostering approaches and experimentally setting up mixed worker groups (Morowitz & Southwick 1987; Calderone & Page 1992; Linksvayer 2006; Linksvayer et al. 2009; Gempe et al. 2012).

Competing Interests

We have no competing interests.

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Table 1. Linear and quadratic selection estimates for behaviors using either reproductive (R) or worker (W) production as the measurement of fitness.

Trait	Estimate (R)	SE	p	Estimate (W)	SE	p
Linear						
Foraging	0.224	0.013	<0.001	0.089	0.005	<0.001
Aggression	-0.059	0.012	<0.001	-0.014	0.005	0.002
Exploratory rate	-0.090	0.010	<0.001	-0.056	0.003	<0.001
Group exploration	-0.013	0.015	0.454	0.133	0.006	<0.001
Colony exploration	-0.134	0.021	<0.001	0.096	0.008	<0.001
Quadratic						
Foraging	0.039	0.037	0.782	0.037	0.017	0.098
Aggression	0.095	0.018	<0.001	-0.212	0.011	<0.001
Exploratory rate	0.119	0.019	<0.001	0.110	0.007	<0.001
Group exploration	-0.023	0.033	0.728	0.023	0.016	0.102
Colony exploration	-0.390	0.111	0.002	0.231	0.017	<0.001

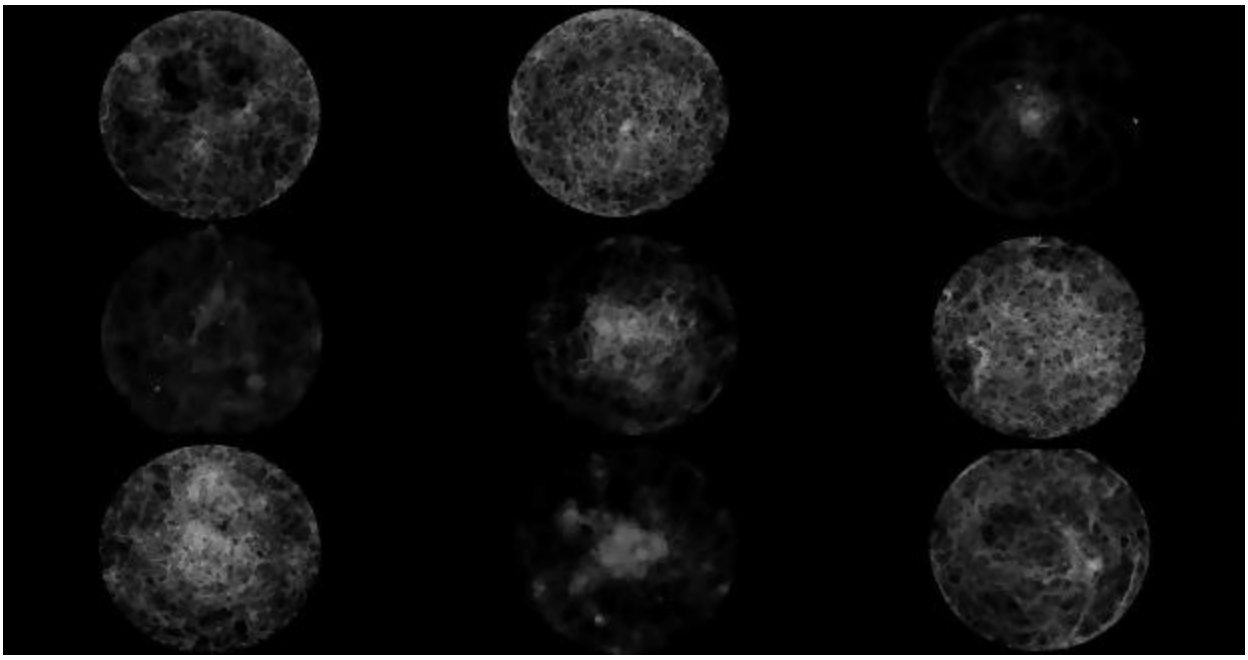

Figure 1. Nine representative plots showing variation among colony genotypes in the exploratory patterns of groups of five foragers. The plots show the tracks (white pixels) of the ants as they explore a novel arena.

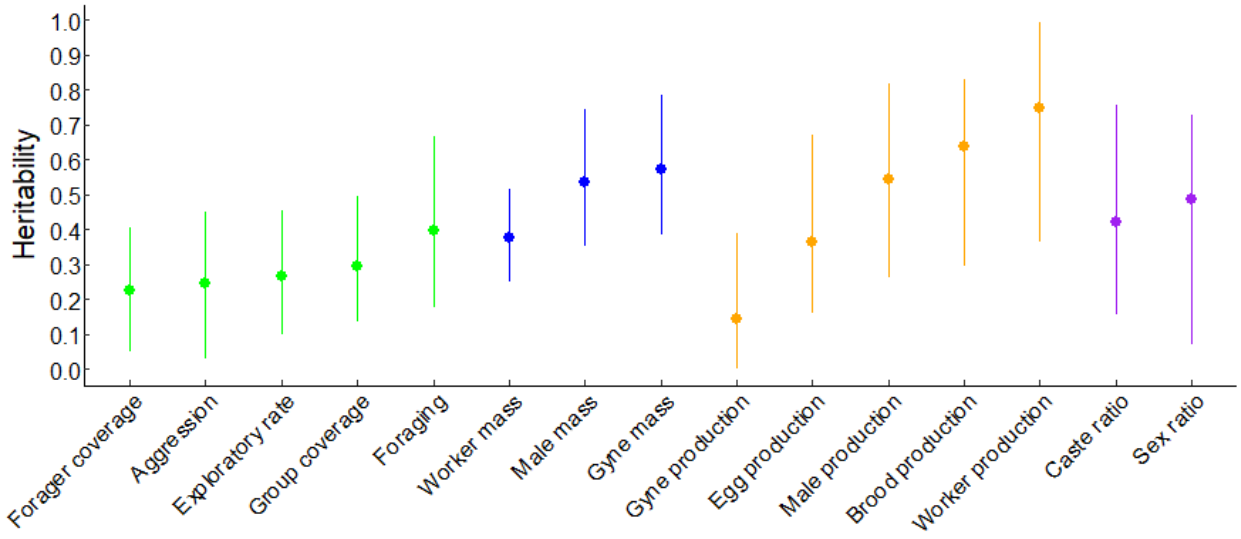

Figure 2. Caterpillar plot of heritability estimates +/- 95% confidence intervals grouped by category. Collective behaviors (green), body mass (blue), colony productivity (orange), and caste and sex ratio (purple) are designated by different colors.

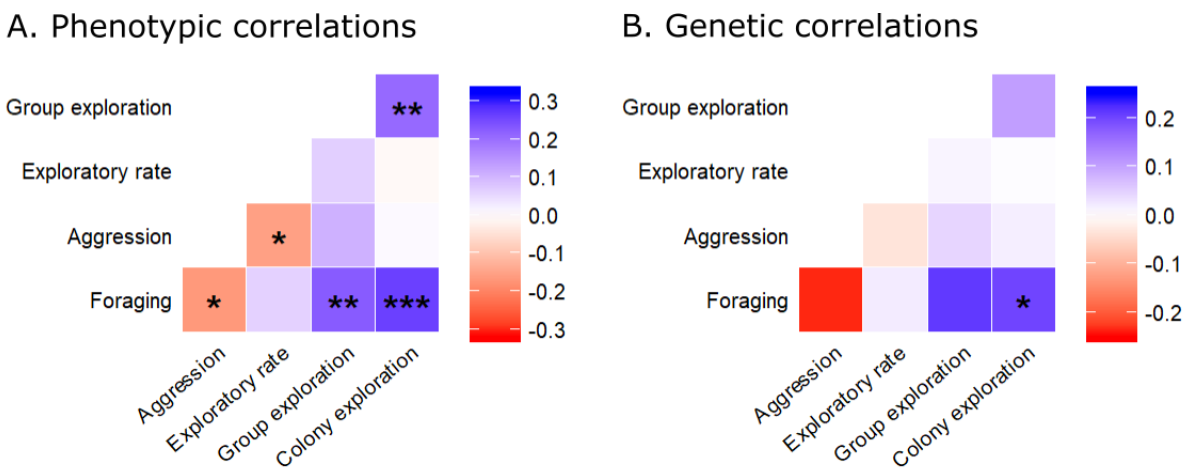

Figure 3. Heatmaps showing phenotypic (A) and genetic (B) correlations between collective behaviors. For the phenotypic correlations, asterisks within cells correspond to p values (adjusted for multiple comparisons; $p < 0.05$ = *; $p < 0.01$ = **; $p < 0.001$ = ***; no symbol indicates $p > 0.05$) and the colors correspond to the magnitude and sign of the Spearman rank correlation coefficient. For the genetic correlations, the asterisk within cells indicates a correlation with 95% CI that do not overlap with zero and the colors represent the genetic correlation estimate, calculated using a Bayesian Markov chain Monte Carlo (MCMC) approach.

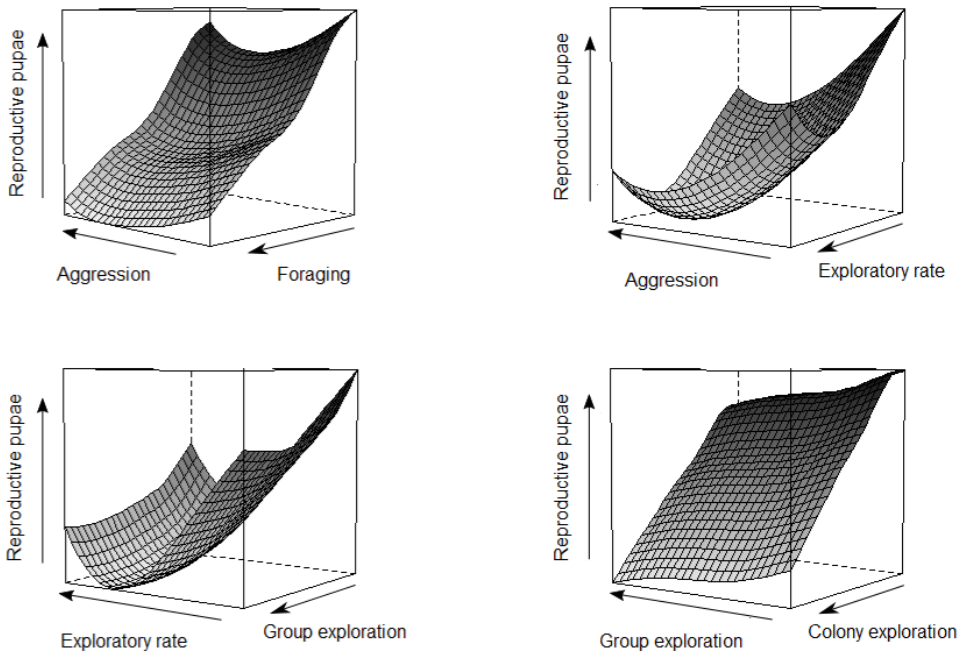

Figure 4. Fitness landscapes showing how variation among colony genotypes for collective behavior corresponds to variation for colony fitness, as measured by the production of new reproductive pupae (see **Supplemental figures 5 and 6** for the full array of fitness landscapes for all combinations of collective behaviors for the production of both reproductives and workers).