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The ontogeny of selection on genetic diversity in harvester ants

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Selection may favour traits throughout an individual's lifetime or at a particular life stage. In many species of social insects, established colonies that are more genetically diverse outperform less diverse colonies with respect to a variety of traits that contribute to fitness, but whether selection favours high diversity in small colonies is unknown. We tested the hypothesis that selection favours genetically diverse colonies during the juvenile period using a multi-year field experiment with the harvester ant, *Pogonomyrmex occidentalis*. We used controlled matings to generate colonies that varied in genetic diversity and transplanted them into the field. We monitored their survival for seven (the 2015 cohort, $n = 149$) and six (the 2016 cohort, $n = 157$) years. Genetically more diverse colonies had greater survival, resulting in significant viability selection. However, in both cohorts survival was not influenced by genetic diversity until colonies were three years old. We suggest that changes in their internal organization enabled colonies to use the benefits of multiple genotypes, and discuss possible mechanisms that can generate this pattern.

1. Introduction

Four decades of field studies on a diverse array of traits and species have provided abundant estimates of the strength of natural selection [1–3]. The overwhelming majority of selection studies examine traits of solitary species, and most studies measure selection once. In social species, some phenotypic characters are analogous to those of solitary species (e.g. group size and growth versus body size and growth). The size of the social group may influence survival or reproduction and result in selection for increased or decreased group size (e.g. [4–6]). In the social insects, estimates of natural selection are rare despite the ecological ubiquity of these species and their importance to evolutionary theory. Quantitative estimates of selection come from studies of the initial stage of the life cycle in ants: males and foundress queens [7–12]. As with many solitary species (e.g. [13]), these studies support the pattern that larger body size is advantageous, whether in competition for mates or in initial colony establishment.

Many phenotypes of social insects are emergent traits that can only be measured at the colony level. Some of these traits show consistent among-colony variation and may contribute to survival and reproduction [14]. Among-colony variation occurs in foraging behaviour, including recruitment intensity [14,15], the temporal pattern of foraging [16,17], the response to relative humidity [18], and in honeybees, the relative collection of nectar and pollen [19]. Studies of colony personality have uncovered variation in colony aggression, whether in response to conspecific non-nest mates or to heterospecific competitors and predators (e.g. [20–22]). Variation in colony level traits is ultimately a product of variation in worker composition which can be generated in several ways, including the proportions of workers belonging to different physical castes, workers of different ages or experience levels or the amount of genetic diversity in the worker force (reviewed in [23]). Many colony level phenotypes change as a function of colony age or size. Worker age and size distributions

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as well as caste distributions are likely to broaden as colonies grow larger (e.g. [24–27]).

Among-colony genetic diversity has been shown to affect traits related to colony fitness in diverse social insect species. A large body of experimental work has documented the advantages of diverse colonies in resisting disease [28,29], increasing foraging efficiency and food retrieval [30–32], increasing division of labour (reviewed in [23]) and maintaining colony homeostasis [33]. In honeybees, a well-studied model system, the genetic diversity generated by high levels of multiple mating is correlated with higher performance in a range of phenotypes (e.g. [29,34–36]). Many of these effects have been shown to arise through genetic specialization [37,38]. Virtually all of this work has been carried out in reproductively mature colonies, or in species that reproduce by colony fission and never have small colonies, such as honeybees or army ants [39,40]. An exception is a study manipulating genetic diversity in small colonies of Argentine ants [41], which found that colony size, but not genetic diversity, affected short-term task performance and reproductive output. However, in many species of social Hymenoptera, colonies are founded by a queen who may live for one or a few years, or for decades [42]. Compared to fission-founding species, colonies are initially much smaller that they will be at reproductive maturity. How selection acts on genetic diversity during ontogeny in such species is of interest given that colonies undergo considerable change in size and structure during this period [24].

We tested the hypothesis that selection favours genetically diverse colonies during the early stages of colony founding using the harvester ant, *Pogonomyrmex occidentalis*. Previous work on this species demonstrated a positive correlation of genetic diversity with growth [43,44] and with the onset of foraging [16], leading to greater food intake [45]. Colonies of this species are founded by a single, multiply mated queen, and over several decades may grow to contain up to 20 000 workers [46]. To address the limitations of short-term studies, we measured selection in each of two independent cohorts over several years. In *P. occidentalis*, incipient colony survival depends on the size of the founding queen [11], and is a period of extremely high mortality (98% of colonies do not survive). We experimentally varied the number of matings by queens and therefore the genetic diversity among workers, and bypassed the incipient colony stage by growing colonies in the laboratory for approximately ten months. We transplanted these small colonies into the field and followed their survival for up to seven years. We measured the strength of viability selection on genetic diversity in each year for each transplant cohort to determine how the intensity of selection varied over time and with colony age.

2. Material and methods

We transplanted 306 experimentally constructed colonies over two years (2015–2016) and subsequently tracked their survival through activity censuses. The methods for initiating, raising and transplanting the colonies are described in detail elsewhere [47]. Briefly, we used watering to induce the release of reproductives [48], collected and sorted these, and then mass-mated females from individual colonies with males from at least ten different source colonies for 30–60 min [49]. Different colonies were used as the sources of queens and males. Mated queens were shipped to the University of Houston and reared under standard conditions for approximately ten months, bypassing the incipient colony stage, before the entire colony was returned to the field for transplanting. Colonies were transplanted into an area adjacent to our long-term study site (F&W TRW MA-02 and MA-03 in BLM/CO/PL-15/016; https://eplanning.blm.gov/public_projects/lup/55944/67731/73684/4._GJFO_Approved_RMP.pdf) in Mesa Co., Colorado. Before colonies were returned to the field, we removed a worker sample to determine the number of times that the queen had mated. Not all experimental colonies were considered large enough to transplant. We transplanted all colonies that had at least 100 workers after the sample for genetic analysis was removed (61.5% of experimental colonies in 2015, and 62.2% of colonies in 2016). The initial size of transplanted colonies ranged from 140–360 workers.

We transplanted 149 colonies in 2015, and 157 colonies in 2016, and monitored survival of these colonies through summer 2021. During the first summer, we monitored activity daily [47], and for the remainder of the experiment we monitored activity weekly from mid-May through mid-August, with an additional set of censuses in mid-September during the first five years. A colony was scored as surviving in a given year if activity was observed at any time during the monitoring period. All surviving colonies were still pre-reproductive in 2021.

We determined the number of times that each queen mated by genotyping a sample of 24 workers from each colony using four highly polymorphic microsatellite loci [44]. All queens of colonies transplanted in 2015, and most queens of colonies transplanted in 2016 were produced by colonies that had been genotyped previously [16,44], which facilitated the identification of maternal alleles. In 2016, some colonies were produced from field-collected queens that had mated naturally. We also determined the genotype of the queen of each male source colony. This enabled us to confirm patriline identity within experimental colonies. The number of patrilines (unique multi-locus haploid genotypes after the queen alleles were accounted for) was equal to the minimum number of mates. The controlled matings produced a range of mate numbers comparable to field-assayed colonies (2015: 5.09 ± 2.05 (mean ± s.d.), range 1–12, $n = 149$; 2016: 6.01 ± 1.68, range 1–11, $n = 157$). We used the number of matings by the queen as the measure of genetic diversity. The number of genetic lineages in the colony provides a clear link to mechanisms that invoke genetic specialization. However, we also calculated the effective mate number and the degree of relatedness and show that the results below are unchanged (see electronic supplementary material).

Attributes of the queen, such as her size and matriline may have affected the number of times she mated. We measured the size of the queen after mating [47] and asked whether size or matriline were correlated with mate number. Mate number was unrelated to queen size (2015: $r = 0\ 04$, $p > 0.6$, $n = 138$; 2016: $r = -0.03$, $p > 0.6$, $n = 156$) and to matriline (GLM 2015: $F_{19,116}$=1.25, $p = 0.23$, $n = 136$; 2016: $F_{12,73} = 1.40$, $p = 0.18$, $n = 86$). We also asked whether matriline or mate number affected growth in the lab by measuring whether they were correlated with colony size (number of workers) at the time of transplanting. Neither mate number (2015: $r = -0.03$, $p > 0.7$, $n = 142$; 2016: $r = 0.06$, $p > 0.4$, $n = 156$), nor maternal lineage of the queen (GLM 2015: $F_{19,115} = 1.15$, $p = 0.31$, $n = 135$; 2016: $F_{14,68} = 1.44$, $p = 0.16$, $n = 83$) was correlated with colony size.

We calculated the effect of mate number on survival by using a Cox-proportional hazards model (SAS 9.4 proc phreg, [50]) that allowed us to estimate the effect of a covariate (mate number) on survival. We estimated the effect of maternal lineages on survival, for those lineages with at least three colonies, using a Kaplan–Meier test for heterogeneity of survival functions (SAS 9.4 proc lifetest [50]). We estimated the directional selection gradient by regressing colony fitness (survival, measured as 0 or 1) on colony phenotype (the number of matings by the queen) for

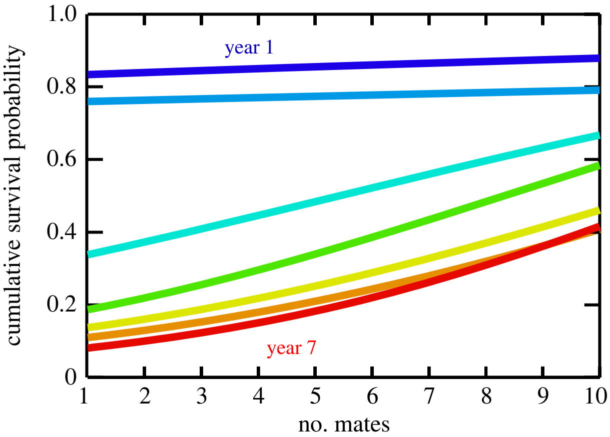

Figure 1. Plots of the logistic regressions of survival on mate number for 7 years of the experiment. Due to ongoing mortality of transplanted colonies, the curve for each subsequent year falls below the curve for the previous year. For years 1 and 2, the regression of survival on mate number was not significant (year 1, $p = 0.19$; year 2, $p = 0.39$). In years 3–7 the logistic regressions were significant (year 3, $p = 0.012$; year 4, $p = 0.002$; year 5, $p = 0.0067$; year 6, $p = 0.0089$; year 7, $p = 0.028$). In years 1–6 the regressions include both the 2015 and 2016 transplant cohorts ($n = 306$ colonies). Only the 2015 cohort was available for the year 7 regression ($n = 149$ colonies). (Online version in colour.)

each year of the study. The standardized linear regression coefficient is the directional selection gradient on viability. Because fitness only has two values, we also performed logistic regression of fitness on phenotype for each year of the study. Unlike linear regression, in logistic regression, the slope is not constant across the phenotypic range (mate number categories). We used the method of Janzen & Stern [51] to calculate the gradient experienced by each mate number category separately, and then averaged over all categories to estimate the selection gradient for the population.

3. Results

Greater genetic diversity increased colony survival. Mate number was a highly significant covariate in the survival analysis, both for six years of survival (Cox-proportional hazards model: likelihood chi-square = 8.94, d.f. = 1, $p = 0.003$, $n = 302$ colonies) and for 7 years of survival (the 7th year represents data from the 2015 transplants only: likelihood chi-square = 6.53, d.f. = 1, $p = 0.011$, $n = 145$ colonies). Sample size in this analysis differed slightly from the total colonies transplanted because four colonies had to be censored within the experiment. The results obtained from analyses that used other measures of genetic diversity (effective mate number, relatedness) gave the same results (electronic supplementary material). Maternal lineages did not differ in survival (Kaplan–Meier heterogeneity of survival curves, log-rank chi-square = 39.4, d.f. = 32, $p = 0.17$; Wilcoxon chi-square = 36.0, d.f. = 32, $p = 0.29$).

The fitness benefits of genetic diversity were delayed. During the first two years mate number did not significantly affect survival, but beginning in year three, colonies with higher genetic diversity had significantly higher survival (figure 1). In years 3–7, the logistic regression of survival on mating frequency was significant. The selection gradient was effectively zero during the first 2 years after transplanting, abruptly increased in year three, and remained significant during the remaining years of the experiment (figure 2). Regardless of whether the selection gradient was estimated using linear regression or logistic regression the results were essentially identical (the correlation between the two measures was 0.998). After three years, the selection gradient stabilized at about 0.15–0.20.

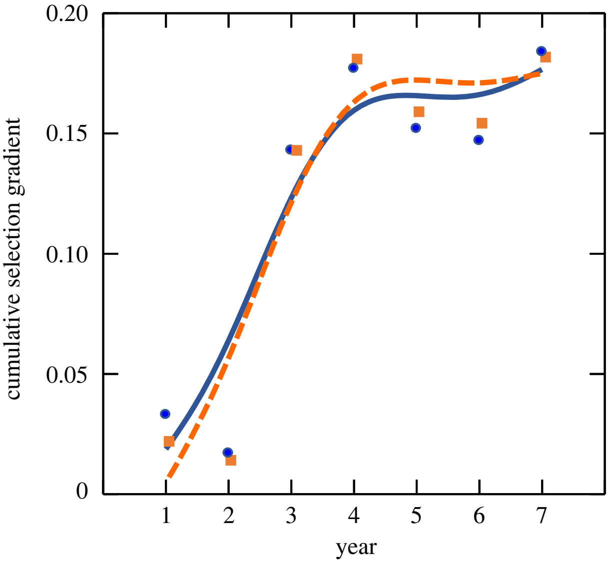

Figure 2. The cumulative realised selection gradient through time. The blue symbols (circles) and solid line show the selection gradient calculated from the standardized linear regression of survival on mate number, while the orange symbols (squares) and dashed line show the average gradient of the logistic regression of survival on mate number [51]. The red symbols are shifted slightly to the right to minimize overlapping points. The lines are a density-weighted least square smooth to indicate the general pattern of change in the selection gradient. The significant regressions for years 3–7 (figure 1) result in a selection gradient of 0.15–0.20. (Online version in colour.)

The change in selection intensity may be due either to an environmental factor, or to a change in colony properties over time. If some factor in the external environment was responsible for the changing intensity of selection, the colonies from the two transplant cohorts should respond synchronously, but the response will occur at different ages. If the change in intensity was due to a change in colony state, then we expect a concordant pattern when the cohorts are age-matched. We plotted the change in the logistic regression coefficient as function of colony age (figure 3*a*) and year of the study (figure 3*b*). Changes in the intensity of selection were significantly positively correlated with colony age ($r = 0.78$, $p = 0.033$), while the correlation between the date matched cohorts was negative ($r = -0.19$, $p > 0.5$; one-tailed tests because we predicted a positive correlation).

Directional selection on colony genetic diversity increased the average diversity over the course of the experiment (figure 4). The average number of patrilines in mature colonies in this population (6.15 ± 1.55 [mean ± s.d.], $N = 150$ colonies) was measured in an earlier study [52]. At the time of transplanting the experimental colonies had significantly fewer patrilines (5.57 ± 1.92) but approached the mature colony mean by the end of the experiment (year 7, mean = 6.12 ± 1.54).

4. Discussion

This study demonstrates an ontogenetic change in the value of genetic diversity for viability selection in harvester ants.

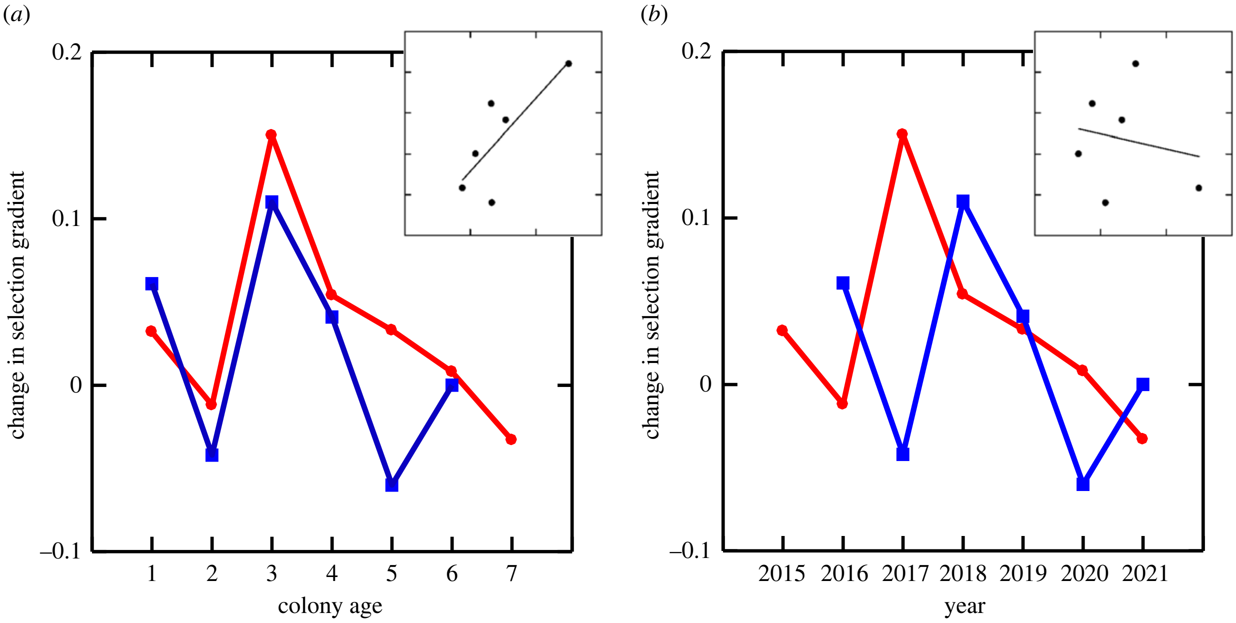

Figure 3. The annual change in the selection gradient for the 2015 cohort (circles, red lines) and the 2016 cohort (squares, blue lines) as a function of (*a*) colony age and (*b*) year of the experiment. The insets show the change in the selection gradient of the 2015 cohort (*y*-axis) plotted against the 2016 cohort (*x*-axis). (Online version in colour.)

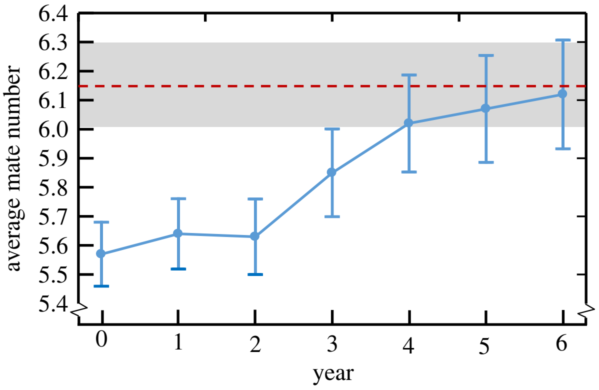

Figure 4. The mean mate number of surviving colonies during six years of the experiment. The red dotted line and shading indicates the average number (and se) of patrilines for mature colonies in this population (6.15 ± 1.55 s.d., $n = 150$ colonies, 53). Year 0 is the average number of patrilines for colonies that were transplanted (5.57 ± 1.92, *t* test of difference with population mean, $p = 0.0006$, $n = 306$ colonies). In subsequent years the number of patrilines increased toward the population mean (year 6, 6.12 ± 1.54, $p = 0.90$, $n = 68$). Note that the *y*-axis is truncated. (Online version in colour.)

Consistent with results from mature colonies, highly genetically diverse juvenile colonies have a substantial fitness advantage over less diverse colonies, suggesting that the benefits to multiple mating manifest within the first few years of colony life. The persistent selection gradient on mating frequency of approx. 0.15 is approximately twice as great as the median gradient for life-history characters [53].

The delay in the onset of selection until a colony was approximately three years old, was a striking, and unexpected pattern that occurred in both transplant cohorts, and strongly suggests that an internal change in the colony is responsible. The initial transplant populations had a broad range of mate numbers, and we expected that more diverse colonies would have an advantage from the beginning. Although very small colonies may not express the full range of genetic diversity that will be present later, changes in genetic diversity over the course of our experiment are unlikely to explain our results. We measured diversity in ten-month-old colonies, ranging in size from 140 to 360 workers, which should have enabled us to estimate mate number accurately. It is more likely that emergent properties that resulted from increased colony size, synergizing with genetic diversity, were responsible for the patterns we observed.

Over the first several years of life, several concurrent changes in colony structure and organization occur (e.g. [54]). The simplest is that colonies become larger, the number of workers in the colony increases. Accompanying increased colony size are changes in the organization of colony behaviour (e.g. [55]). Complex nest architecture can structure the organization of activities within the nest [54,56]. In small colonies of social insects almost all workers interact with each other [57], while in larger colonies, the network of interactions among workers often becomes more modular, with interactions not distributed equally among individuals [58,59]. The increased modularity of organization in larger colonies appears to be a property of networks generally, rather than social insect colonies specifically (e.g. [60]).

Larger colony size can result in more complex patterns of division of labour. Because most individuals in small colonies interact with each other [57] the needs of the colony can be assessed by most workers [61] and division of labour is minimal. As the colony grows, workers may show increasing specialization on tasks and the degree of division of labour increases. Measures of division of labour increase both as a theoretical prediction [58,62] and in empirical results [63–67]. Efficiency may also increase as a function of colony size: in *Pseudomyrmex*, conflicts over work resolve faster in larger colonies [68].

Genetic specialization may facilitate more substantial division of labour. Specialization has been investigated primarily in polyandrous colonies, but it is also expected in colonies with multiple queens (e.g. [66]). The association of behavioural task with genetic lineage in social insects has a long history, beginning with pioneering work on the response to disease in honeybees [69–71]. These early results have been extended to additional tasks in honeybees such as guarding behaviour [70], and pollen and nectar foraging [19] and in ants to specialization on foraging versus waste management [72,73], rescue behaviour [74], resistance to fungal diseases [75,76] and foraging [16]. Although division of labour can occur at the very beginning of colony life [77], as specialization becomes more extensive in larger colonies, there is a greater association of specific patrilines with particular tasks which in turn leads to greater performance by colonies with higher genetic diversity [16,33,36,38,78,79]. This association may explain why the lineages of social insects whose queens mate multiply have colony sizes that are larger on average than those that mate a single time [80].

The modularity of network organization, whether a function of nest organization or of the spatial separation of different aged workers, [81,82], also has the effect of reducing pathogen transmission [83,84]. Network structure can respond to changing conditions in the nest: modularity in the ant *Lasius niger* increased when colonies become infected with a pathogen [85]. In larger social groups, increased network modularity reduced disease transmission [86,87] but the extent to which genetic diversity might amplify this effect has not been tested.

Colonies with solitary foundresses undergo an inevitable delay between colony initiation and the realization of the genetic diversity from multiple mating. Young colonies experience a window of vulnerability because of their small size, when survival or performance [41] may be largely independent of genetic diversity. Small colonies may be more affected by competition from conspecifics or heterospecifics (e.g. [88]) or environmental factors [47]. As colonies grow, they are able to realise more of the benefits of increased specialization, network modularity and architectural complexity. The colonies of all social insect species will experience these advantages as they grow, but genetic diversity interacts synergistically with size, amplifying the advantages of sociality.

The colony phenotypes that result from genetic diversity are emergent characters that lack equivalents in solitary organisms. The most analogous comparison are studies that quantify the advantages to outcrossed versus selfed progeny in plants (e.g. [89]). The proposed mechanisms by which genetically diverse colonies increase their probability of survival rely on colony growth: an increasing number of workers and of genotypes. Our results parallel studies of selection on body size in solitary organisms that overwhelmingly demonstrate an advantage to larger size [3]. They also deviate from that pattern, by documenting a delay in the selective advantage of diversity, a delay that was detected only because we measured survival for several years. Multiyear studies of selection are relatively uncommon but may yield insights that single-episode studies cannot.

Data accessibility. Additional data are available in the Dryad Data Repository (doi:10.5061/dryad.xgxd254jc) [90].

Authors' contributions. D.C.W.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, validation, writing—original draft, writing—review and editing; B.J.C.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, validation, visualization, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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