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The benefits of being big and diverse: early colony survival in harvester ants

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Abstract. In sessile organisms such as plants and benthic invertebrates, founding propagules typically suffer extremely high rates of mortality due to both extrinsic and intrinsic factors. Many social insect species share similarities with these groups, but factors influencing early colony survival are relatively unstudied. We used a field experiment to measure the importance of environmental quality relative to intrinsic colony properties in the harvester ant, *Pogonomyrmex occidentalis*, by monitoring the survival of 584 experimental colonies. We measured survival of transplanted colonies over four months in each of three years (2014–2016) at a site in western Colorado. Colony survival was primarily determined by colony features. Multiple mating by the queen and larger colony size at the time of transplant increased survival, but queen size, maternal lineage and the composition of plant species in the vicinity of the colony did not. Food supplementation increased survival significantly when natural food was scarce, but was not consistently beneficial, in contrast to predictions. Our results emphasize the general importance of rapid growth and early attainment of large size in the survival of sessile species. However, attributes specific to ants that are a consequence of their sociality also strongly affected survival. Colonies with multiply-mated queens were more likely to survive over a wide range of circumstances, highlighting the importance of this trait even at the early stages of colony life.

Key words: habitat quality; juvenile mortality; multiple mating; Pogonomyrmex; *recruitment; transplant.*

Introduction

Events that occur during the juvenile stage often have profound and life-long ecological and evolutionary consequences. High mortality or survival of juveniles can alter the age structure, cohort size, and population dynamics of long-lived species (e.g., fish: Chambers and Trippel 2012; plants: Eriksson and Ehrlén 2008). The spatial pattern of juvenile establishment may affect the density of competitors, or reproductive partners or the intensity of predation in species with sessile adults. The juvenile stage is frequently characterized by high mortality (Gosselin and Qian 1997), and is often the time in the life cycle when the opportunity for selection is the greatest (Reiss 2013). Food availability at early stages may affect adult size with consequent effects on fecundity (e.g., Taborsky 2006), mating success (e.g., Barrett et al. 2009) and in species with size-based demography, age of maturity (e.g., Gosselin and Qian 1997). In many cases, early food deprivation cannot be compensated for by later food abundance (e.g., Boggs and Freeman 2005).

Early stage size and survival will be influenced by both extrinsic and intrinsic factors. Annual variation in environmental quality, such as drought or food availability, will affect recruitment and juvenile survival leading to variation in cohort size (e.g., Biro and Post 2007, Lopez and Kursar 2007). Early mortality is influenced by annual variation in the physical environment in plants (Wright et al. 2005), corals (Gleason 1996), and other marine invertebrates (e.g. barnacles Caffey 1985, Jenkins 2000).

Intrinsic factors can also affect early stage survival. Parental investment leading to larger propagules may enable establishment in locations where survival would not otherwise be possible. For many animals, size at birth or hatching influences survival and reproductive fitness (Lindström 1999, Krist 2011). For fish, the evidence also suggests that larger eggs are more likely to hatch and have an initial survival advantage as fry (e.g.,

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Marsh 1986). In plants, larger seed size often influences the early establishment of seedlings (Moles and Westoby 2004). Within a species, larger seed size may be correlated with larger seedling size, greater survival, and larger adult size and corresponding seed production, but also potentially longer time to maturity (Moles and Leishman 2008).

Many species of ants share life history traits with plants and benthic invertebrates, including small founding propagules, high mortality during the colony initiation stage, and a relatively sessile adult stage that can be long lived (Hölldobler and Wilson 1990). Comparisons among these groups would provide a robust test of the general importance of factors that select for this shared life history. Previous work has suggested that aspects of environmental quality, including the availability of food resources, the level of disturbance, and density and proximity of competitors, can affect the establishment and juvenile survival of ant colonies (e.g., Adams and Tschinkel 1995, Kaspari and Valone 2002, Tschinkel and King 2017).

Comparisons among species with similar life histories, but belonging to unrelated groups, enable us to identify general ecological and evolutionary processes. Juvenile survival processes have been well studied in plants and in certain marine invertebrates, but have been much less studied in ants. However, ants are also distinguished by their sociality. Characteristics of the colony's queen, as well as colony level phenotypes that result from the composition of the colony's workers, may affect initial establishment (Wiernasz and Cole 2003, Enzmann and Gibbs 2014), as well as survival in older colonies (Cole and Wiernasz 1999, Wiernasz and Perroni 2004). These include both the number and distribution of workers belonging to different physical castes (Yang and Martin 2004), and the genetic diversity of the worker force (Wiernasz et al. 2004, 2008). The extent to which the social environment, which comprises both strict maternal effects as well as effects produced by interactions with other members of the social group, influences colony establishment and survival is unknown. Determining the importance of the social relative to the physical environment for survival during this critical stage of colony establishment will provide insight into the demography of this ecologically important group.

We measured the effect of variation in colony properties and environmental factors on early colony survival in the harvester ant, *Pogonomyrmex occidentalis*, using a multiyear field experiment. We directly manipulated those factors that we predicted would have the greatest effect on colony survival: additional food, mate number, and queen source colony, but we also quantified initial colony size and the vegetation composition in the nest area. Food supplements are known to affect several aspects of colony performance in ants, especially reproductive output (e.g., Deslippe and Savolainen 1994, Kaspari and Valone 2002, Foitzik et al. 2004, Smith 2007, Sorvari and Hakkarainen 2007, Seal and Tschinkel 2008). Mate number is positively correlated with disease resistance (Wilson-Rich et al. 2009) and colony performance (Oldroyd and Fewell 2007). In *P. occidentalis*, the number of times that the queen mates increases growth in mature colonies; faster growth is correlated with survival (Cole and Wiernasz 1999, Wiernasz et al. 2004). Although the advantages of additional food and higher genetic diversity in mature colonies are well established in ants, their importance for the survival of juvenile colonies is unstudied. The genetic diversity that arises from multiple mating may not be fully present in small colonies. Larger or higher quality queens may be more be more likely to successfully found colonies (Wiernasz and Cole 2003), but the degree to which these benefits persist is unknown. We hypothesized that food addition and multiple mating by queens would increase colony survival, and that source colonies which produced larger queens would have daughter colonies with higher survival.

Methods

Transplanted colonies were located adjacent to our long-term study site northwest of Fruita CO, USA (Wiernasz and Cole 1995) (Appendix S1: Fig. S1). Colonies were constructed using directed mating, grown in the laboratory under standardized conditions for 10 months, transplanted into preselected locations, subjected to a food manipulation and monitored for survival. We briefly describe the methods for constructing, transplanting, and monitoring colonies, quantifying vegetation composition and spatial patterns of mortality, and statistical analyses. More detailed methods are provided in Appendix S1.

Experimental colonies

Colonies were constructed by controlled matings of unmated females paired with one male (singly mated) or placed with 200–300 males from several different colonies (multiply mated) using the procedures described in detail in Wiernasz et al. (2014). We did not verify mate number, however the mass mating technique generated multiply mated queens previously (Wiernasz et al. 2014). Sperm volume was not controlled in this study, but a single mating transfers over a million sperm (Tschinkel 1987), sperm depletion during the course of the experiment is extremely unlikely. In 2015, an early reproductive flight ended colony construction. We collected naturally mated foundresses to increase the number of experimental colonies. These queens were treated as multiply mated, and survived similarly to the experimentally mated queens. Queens were sent to the University of Houston and allowed to establish colonies and grow until they were transplanted the following spring. The size of queens after mating was measured in each year immediately after they arrived in Houston (see Appendix S1).

Transplant procedures

Colony size (number of workers) was determined before colonies were sent back to the field. Upon arrival in Colorado, each colony was transferred to a 1 L cardboard container filled with local soil, and then transplanted into previously mapped sites (for details of colony preparation, see Appendix S1). In every year of the experiment, the number of colonies precluded all of them being transplanted on the same day. In 2014 we transplanted a total of 82 colonies (June 15–16), in 2015 we transplanted 276 colonies (150 on May 5–7, 126 on May 24–25) and in 2016 we transplanted 226 colonies (148 on May 5–7, 78 on May 22–23). We refer to each transplanted group of colonies as a transplant set. In 2014 and 2015, transplanted colonies were separated by 10 m or more. In 2016, colonies were separated by at least 7 m in all directions (see Appendix S1). We initially laid out colony sites as a grid, then adjusted locations to prevent colonies from being transplanted into sites where they do not naturally occur (e.g., under shrubs). Our criteria for microsite selection were based on ~6,000 locations of colonies in our long-term study site.

Experimental treatments

We manipulated mate number and one aspect of environmental quality in a balanced two-way design. To mimic the effect of higher quality sites, supplemented colonies received a twice weekly food addition of 1.5–2 g of organic mixed grains (cracked wheat, oats, barley, rye, millet, flax); control colonies received no additional food. The goal of food supplementation was to double the food intake of colonies. The amount of each seed addition was equivalent to 70–80 additional successful forager trips.

The queens of experimental colonies came from known source colonies (matrilines) which enabled us to address lineage quality effects. We balanced the food supplement treatment across the mating treatment category and across matrilines. Colonies were then randomly assigned to transplant locations to break any possible correlation between colony treatments and site characteristics. A few colonies were not very active and their food was taken by a neighbor. The food treatment category of these colonies was reassigned to reflect this. Of the total of 584 colonies, 285 (49%) received food and 226 (39%) were singly mated.

Colonies varied in size at the time of transplanting. Although colonies were all "founded" at the same time and treated similarly, colonies differed in their growth rate while being reared in the laboratory. Colony size at transplant varied across years ($F_{2,453} = 7.1$, $P < 0.001$), and was somewhat influenced by maternal lineage ($F_{39,453} = 1.35$, $P = 0.08$), but not by mating frequency ($F_{1,453} = 0.07$, $P > 0.7$). We included the number of workers at the time of colony transplant as a treatment effect in the survival analyses.

Monitoring survival

We used worker activity as a proxy for colony survival. All transplanted colonies were monitored for three days immediately following transplanting. Ten colonies were never active, and were assumed to have died during transplanting. In 2014, colonies were monitored daily from the date of transplant. However, in 2015 and 2016, transplanted colonies were split into two sets. This caused a gap in the daily monitoring for the early transplant sets; daily monitoring began on June 3 for both years (all remaining colonies survived to this date). The start of the survival period is the transplant date. Colonies were observed three times each morning, from 07:00 h to12:00 h or until activity stopped (see Appendix S1 for details). Living colonies were not active every day. Colony activity was monitored until mid-August, and then again during a five-day period in September, which was the final date at which activity could be observed. We inferred the date of death by noting the last date on which colonies were known to be active. A colony may have been dormant (or unobserved) on some days, but if it was seen subsequently, it was alive in all earlier censuses. Colonies that were known to be alive in the final census are regarded as right censored (that is, they survived beyond the end of the experiment, Cox and Oakes 1984).

Vegetation

Although the location of colonies by experimental treatment was randomized, individual nest sites still differed in physical and biological characteristics. We measured the composition of vegetation at each transplant site (see Appendix S1). Differences in vegetation may reflect differences in the available food resources for the ants as well as other differences such as soil type, exposure and slope. We quantified the variation in vegetation in the neighborhood of each transplanted colony as the principal component score (see Appendix S1 for details). For all years, we used the scores for the first two principal components as covariates in the survival analysis (for PC loadings see Appendix S3).

Statistical methods

Survival analysis of transplants.—Survival analyses were conducted using SAS 9.4 (SAS Institute Inc. 2013). We used a Kaplan–Meier test of homogeneity to determine whether survival differed among sets (set 1 from 2014, sets 2 and 3 from 2015, and sets 4 and 5 from 2016). The results clearly demonstrated a year and/or transplant set effect, so in subsequent analyses we stratified the survival analyses by year, and nested transplant set within year.

We analyzed survival using a Cox proportional hazards model (PROC PHREG in SAS 9.4, see Appendix S1 for details). The inferred date of death (or

the latest date when observations of activity are made for censored data) was the response variable. Our experiment incorporates controlled variation, but also uncontrolled variation in factors that could be important for survival. From previous studies on ants, we had *a priori* expectations that some treatments (multiple mating, additional food) would increase colony survival, others (matriline) might differ in survival, although the differences would not be predictable. Variation in other factors likely to affect survival (colony size at transplant, vegetation composition) emerged during the experiment. Finally, the experiment was conducted over multiple years. For these reasons, we analyzed our data with a stepwise elimination procedure.

Stepwise procedures, particularly when they involve a large number (>10s to 100s) of independent variables, often find false-positive effects and if the independent variables are correlated, the final model will likely not include the most important factors (Whittingham et al. 2006). Because we began with three independent variables, stratified by year, these concerns were lessened. We also assigned colonies randomly to experimental treatments ensuring that the independent variables were uncorrelated. A significant issue with a stepwise procedure is the inflation of *P*-values (Forstmeier and Wagenmakers 2017). We dealt with the issue of cryptic multiple testing by noting the number of tests that were performed at each step in the analysis and then used the total number of tests performed to form a protected alpha value using the Benjamini–Hochberg procedure (with a false discovery rate of 0.25). We performed 29 total significance tests in the stepwise procedure, which gave an adjusted α value of 0.008. We began with a fully saturated model including all of the main effects (mating status, food addition status, colony size at transplant) and interactions, as well as all effects using transplant set within year as an interaction. We specified that effects would be eliminated when the *P*-value (in joint tests) > 0.10. We enforced a hierarchical elimination procedure such that no treatment of lower order could be excluded if the interaction effect was included in the model.

Effects of queen size, matriline and vegetation on survival.—We analyzed the effects of local vegetation, the size of queen and the queen's lineage (matriline) on survival separately from the main survival analysis. There were yearly differences in vegetation, as well as differences among transplant locations that confounded the vegetation scores with the year of transplant. We asked whether vegetation composition influenced survival beyond the experimental treatments during a given year (Appendix S3).

Because queen size varies among matrilines and years (Wiernasz and Cole 2018), we performed a survival analysis including queen size as a covariate for each year separately. In 2016, the mass measurements of collected foundresses differed from queens that were mated in directed matings. This is likely to be due to foundresses having lost water while digging compared with experimental queens that were maintained under hydrated conditions.

We tested for the effects of queen lineage on survival in separate analyses. Forty colonies were the source of queens in this study, although not all colonies contributed queens in all years. The source colonies for the natural foundresses in the 2016 transplants were unknown. The large number of potential interactions with lineage precluded a fully saturated analysis. We tested the effect on survival using year to stratify the analysis, food supplement category, size at transplant and all interactions of those variables in addition to queen lineage.

Spatial analyses of mortality.—We tested for nonrandom spatial patterns in mortality using a randomization procedure to determine if mortality was localized in particular spatial locations due to local differences in vegetation, habitat quality or physical characteristics of the habitat. For each colony that was dead at the end of the summer, we calculated the mean distance to its six nearest dead neighbors in comparison to random expectations (Appendix S1).

Results

Colony survival differed significantly between years (Log-rank $\chi^2 = 68.9$, df = 2, $P \ll 0.001$, Peto $\chi^2 = 81.1$, df = 2, $P \ll 0.001$) and transplant sets (Log-rank $\chi^2 = 78.8$, df = 4, $P \ll 0.001$, Peto $\chi^2 = 91.7$, df = 4, $P \ll 0.001$) (Fig. 1). Survival of colonies transplanted in 2014 was significantly worse (50%) than those transplanted in 2015 and 2016 (70% and 87% respectively, Kaplan–Meier, Log-rank $\chi^2 = 52.8$, df = 1, $P < 0.0001$). In 2015, the second group of colonies that was transplanted survived significantly worse than the first set (63.1% compared with 76.6%, Kaplan–Meier, Log-rank $\chi^2 = 8.1$, df = 1, $P = 0.004$). Colonies transplanted in 2016 had the highest rate of survival, and the sets were only marginally significantly different (89.9% compared with 81.8%, Kaplan–Meier, Log-rank $\chi^2 = 5.2$, df = 1, $P = 0.02$). For each of the Cox proportional hazard models below, we stratified the analyses by transplant set within year to account for the heterogeneity of survival rates.

In addition to the planned experimental manipulations (mate number, food supplementation), colonies differed in the maternal lineage of their queen and in queen size. Colonies also differed in size (number of workers) at the time of transplant, reflecting differences in growth rate while in the laboratory. We first present the effect of the experimental treatments, followed by the effects of matriline, queen size and vegetation at the transplant site. Colony survival was affected by both main affects and the interaction between experimental variables and transplant year/set (Table 1). The likelihood ratio

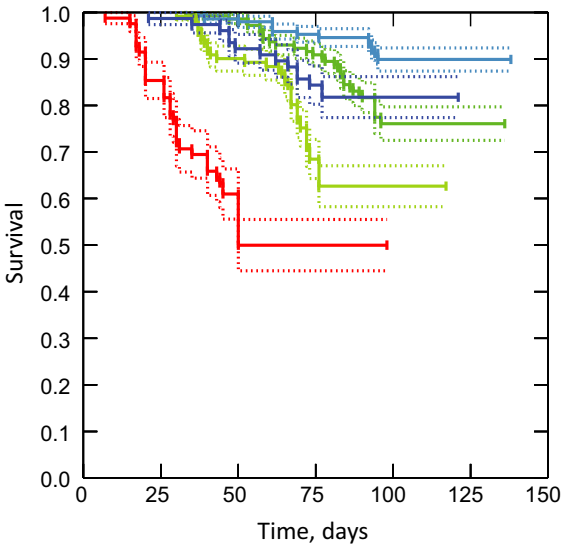

FIG. 1. Kaplan–Meier survival curves for transplant sets: 2014 (red), 2015 (green) and 2016 (blue). The early and late transplant sets in 2015–2016 are distinguished by the longer maximum survival time in early transplant sets.

chi-square for this model was highly significant (χ^2 = 42.43, df = 17, P = 0.0006). We describe the results for the main treatment effects (mate number, food addition, colony size) and the interaction effects separately.

Main effects

Mate number significantly increased survival. Colonies headed by a queen who mated with one male were less likely to survive than ones headed by queens that mated with several males (hazard ratio = 7.24). Overall, food addition did not affect the chance of survival. Colonies that received additional food did not have greater survival than colonies that did not receive food (hazard ratio = 0.67, not significantly different from one). Colonies that were larger at the time of transplant had a greater survival probability than smaller colonies. Relative to the survival of the average colony (223.2 workers), each additional worker increased survival by approximately 1% (hazard ratio = 1.012).

Interaction effects

All of the main effects had significant interactions with other variables. The magnitude and sometimes the direction of the interaction effects depended on transplant set and year. Food supplements had a mixed effect on survival (there was a significant food × set (year) effect; Fig. 2a). Food addition greatly increased survival in 2014; mortality of colonies that did not receive food was substantially higher than if they received a supplement (average log hazard = 0.71). In 2015, food supplements generally decreased survival (average log hazard = −0.50). In 2016, the effect of food was set specific with the early transplant group benefitting significantly more from food addition (average log hazard = 0.45 for the early set and −0.71 for the later set). Food supplementation interacted marginally with mating category, but this also varied among years (Fig. 2a). In 2014, the survival of colonies with multiply mated queens that also received additional food was dramatically greater than those that were not supplemented. In 2015, colonies with singly mated queens that received additional food were at greater risk of dying than colonies that were not supplemented. Colonies with multiply mated queens were not affected. In 2016, there were no significant interactions.

While increased colony size at transplant generally increased survival, there were differences among transplant years (Fig. 2b). In 2014, there was a survival advantage for colonies that were smaller, but this was true only for colonies with singly mated queens. Survival

TABLE 1. Estimated survival statistics for the Cox proportional hazards model.

Effect	df	Wald statistic	P§	Parameter estimate, β†	Standard error	Hazard ratio‡
Mate	1	5.55	0.019§	1.98	0.84	7.24
Food	1	1.03	0.310	−0.40	0.39	0.67
Size at transplant	1	7.71	0.006§	0.012	0.004	1.012
Mate × Food	1	2.96	0.085			
Size × Mate	1	5.38	0.020§			
Size × Set (Year)	4	20.12	0.0005§			
Food × Set (Year)	4	11.02	0.026§			
Size × Mate × Set (Year)	4	8.19	0.085			

Notes: The Wald statistic for the maximum likelihood estimate of the parameters and its significance are shown for all effects retained in the backward stepping procedure.

† For simplicity, the parameter estimates (and their standard errors) are only shown for the main effects. βs are calculated for all effects, but there are multiple β coefficients for the effects with multiple degrees of freedom.

‡ The hazard ratio is exp[β].

§ Indicates results that are significant after Benjamini–Hochberg correction for multiple tests.

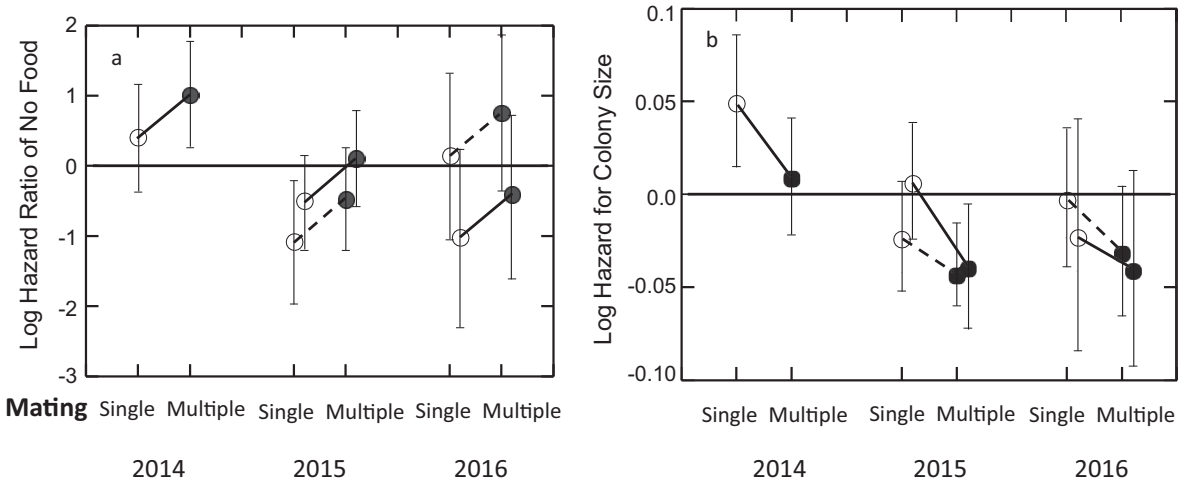

FIG. 2. The log hazard functions. The two transplant sets are shown slightly offset in 2015 and 2016 with the early transplant values connected by a dashed line. Colonies where the queen was multiply mated are shown with filled symbols. (a) The log hazard (and 95% confidence limits) for colonies that did not receive additional food compared with colonies that were supplemented. Values above zero indicate that colonies that did not receive food had a greater probability of dying compared to ones that received food. Values below zero indicate that colonies that did not receive a food supplement had higher survival probabilities. The hazard to colonies that did not receive food was greater for colonies with multiply mated queens than those with singly mated queens. (b) The log hazard for colony size at the time of transplant. The figure shows how the predicted survival hazard changes as a result of having 10 additional workers relative to the average colony size. As most values are below zero, having more workers decreases the hazard (increases survival probability). In 2014, larger colony size at transplant increased the hazard. Mating frequency interacted with colony size; multiply mated colonies enhanced the positive effect on survival (lower hazard).

of colonies with multiply mated queens was unrelated to initial worker number. The difference in hazard for colony size in 2014 accounted for the significant interaction between size and transplant sets. Mate number also interacted with colony size at transplant (Fig. 2b). The survival of colonies whose queens mated multiply was more strongly positively affected by increased colony size than colonies whose queen had mated a single time. Because there was a three-way interaction effect, not all of the effects of mating are identical.

Matriline effects

Maternal lineage did not affect survival (Appendix S2: Table S1). Maternal lineage was the first variable removed in any stepwise analysis. Minor differences in the estimate of other effects can be attributed to differences in sample size due to the fact that maternal lineage is unknown for a subset of the 2016 transplants (the collected foundresses).

Queen size effects

Queen size also did not affect colony survival (Appendix S2: Table S2). In 2015 and 2016, there was no effect of queen size on survival, while in 2014 the effect of queen size on survival was marginal ($P = 0.1$). Queen size was not correlated with the size of the colony at transplant in any year (2014: $r = -0.14$, $P = 0.24$, $N = 77$; 2015: $r = 0.05$, $P = 0.39$, $N = 259$; 2016: $r = -0.02$, $P = 0.76$, $N = 202$), although it was significantly different among maternal lineages in 2015 and 2016, but not in 2014.

Vegetation composition

The effect of vegetation was relatively minimal and varied among years (Appendix S3). No significant vegetation effects occurred in 2015. Although vegetation principal component 1 (PC1) was retained in the final model with a probability of 0.0999 (the cutoff for removal was 0.1) in 2016, it is unlikely that this factor was biologically important. In 2014, there was a significant interaction between food addition and vegetation PC2 ($P = 0.012$, see Appendix S3). Vegetation PC2 primarily contrasted snakeweed (*Gutierrezia sarothrae*), a semiherbaceous perennial often associated with disturbance, with two native perennial species, galleta grass (*Hilaria jamesii*) and globemallow (*Sphaeralcea coccinea*), both are known food plants of harvester ants. Colonies that did not receive food supplements survived better if they were in areas where *G. sarothrae* was relatively common. Colonies that received food survived better when growing in areas where *H. jamesii* and *S. coccinea* were common.

Spatial patterns of survival

The spatial pattern of mortality varied among years (Appendix S4: Fig. S1, Table S1). It was random in 2014, dead colonies were not more clustered than expected by chance (all $z > -1.3$, $P \sim 0.2$). In 2015,

dead colonies were clustered on a relatively large spatial scale: the distance to the first three dead nearest neighbors was not different from random, but the 4th to 6th instances of mortality were significantly closer than expected (4th to 6th: all $z < -2.2$, $P < 0.05$). In 2016, mortality was highly local: the first three dead nearest neighbors were significantly closer to the focal colony than expected, while the 4th to 6th nearest neighbors were randomly distributed (1st to 3rd: all $z < -2.3$, $P < 0.05$). These results suggested that mortality was occurring non-randomly on a larger spatial scale in 2015 than in 2016.

Discussion

Although we observed considerable variation among years in our experiment, some patterns were consistent. Colony growth and rapid attainment of larger size subsequent to colony initiation, was, like post-settlement growth in benthic invertebrates and post-germination growth in plants, critical to colony survival. This pattern is likely to be true of most social insect species with solitarily founding queens (e.g., Marti et al. 2015). Colony growth was affected by both the external environment and the social environment as discussed later.

Multiple mating increased the survival of young colonies directly and also through interactions with food. In social insect species characterized by high levels of multiple mating, some studies have shown that colonies benefit through the effects of increased genetic diversity for resistance to pathogens (Wilson-Rich et al. 2009, Saga et al. 2020) and coping with environmental heterogeneity (Matilla and Seeley 2007, Oldroyd and Fewell 2007). In *P. occidentalis*, mature colonies forage longer when the queen has mated with many males (Wiernasz et al. 2008), increasing food intake and colony growth (Cole et al. 2008). Our results link survival at the initial stages of colony founding to the positive effects of multiple mating on growth in mature colonies (Cole and Wiernasz 1999, Wiernasz et al. 2004). It also suggests why there was a consistent synergistic interaction between multiple mating and food addition.

Mate number also had positive effects on survival that were mediated through colony size: the benefit of having more workers was greater in multiply mated colonies. One proposed advantage of multiple mating is that it can provide a greater range of worker behavioral phenotypes (Fuchs and Moritz 1999, Wiernasz et al. 2008, Huang and Wheeler 2013). Genetically diverse colonies may gain an advantage in dealing with complex environmental challenges. However, this advantage only occurs when sufficient workers are produced, enabling the full range of genetic diversity to be realized.

This experiment did not examine the process of colony founding because of the extremely high mortality (approximately 98% of queens and incipient colonies die, Wiernasz and Cole 2003). In the field, incipient colonies founded by large queens are more likely to survive. The queens in this experiment are likely to have had greater variance in size because they were laboratory reared. However, queen size was not correlated with colony size at transplanting, nor with subsequent colony survival. Our results parallel those of Sales and Toledo (2020), who showed that queen productivity was unrelated to queen size in laboratory-reared *Acromyrmex subterraneus*.

In nature, variation in growth during the initial colony founding stage and after overwintering produces colonies that differ in size. These differences were replicated by our experiment, as a result of variation in growth while in the laboratory. Increased colony size overall had a strong effect on survival with the exception of 2014, when increased colony size in combination with single mating had a negative effect on survival. The small number of colonies in this treatment ($N = 32$) suggests that this result is not biologically meaningful, especially given the robust positive effect of size on survival in the rest of the experiment. Although the overall effect of colony size may be unsurprising – large colonies are more likely to survive than small colonies – it underscores an important point. Any factor that produces faster growth during the earliest stages of colony development will give an additional survival advantage to juvenile colonies. The correlation of greater survival with rapid growth during the juvenile stage is a common feature of the life history of many organisms (e.g., plants: Horvitz and Schemske 2002; fish: Perez-Dominguez and Munch 2010). Indirectly, many studies link differences in juvenile size, which reflect differences in growth, to greater survival (plants: Gilbert et al. 2001; fish: Peterson and Wroblewski 1984; birds and mammals: Ronget et al. 2018).

Although queen size was affected by maternal lineage, neither the size of queens nor their matriline affected the survival of transplanted colonies. This was somewhat surprising, because larger queens are significantly more likely to successfully initiate colonies (Wiernasz and Cole 2003). The advantage of larger queens did not persist beyond the earliest stages of colony founding. These results parallel work in plants, in which early seedlings from larger seeds are more likely to survive (Moles and Westoby 2004), but these effects do not persist to later seedling survival (Moles and Westoby 2006, Moles and Leishman 2008). There is, however, considerable variation among ant species in queen size relative to worker size (Johnson 2002), and it is possible that this effect will not apply to all ant species. We found no detectable differences in the value of queens that come from different genetic lineages after the founding stage has passed, in contrast with observational data from a long-term study of *Pogonomyrmex barbatus* (Ingram et al. 2013).

Environmental factors that can vary among years include temperature and precipitation, food availability, and the density of competitors, pathogens and predators. We manipulated some, but not all, of these and found that colony survival varied significantly among years. Annual cohorts of queens attempting to establish

colonies will face very different probabilities of success (for example, overall survival in 2014, 50%, was much lower than in 2015, 70% or 2016, 87%). The survival rates among experimental colonies paralleled those of one-year-old colonies surviving to their second year at our long-term study site (2014: 29%, 2015: 41%, 2016: 64%; Cole and Wiernasz, *unpublished data*), suggesting that our experimental transplants are experiencing conditions similar to naturally founded colonies.

Years may vary in several ways that could influence the probability of successful colony founding. Spring precipitation determines the abundance of annual plants and the productivity of both annual and perennial plant species whose seeds are the primary food of *P. occidentalis*. Rainfall is correlated with temperature: years with low precipitation are also hotter, which can increase the risk of death to foragers (Friedman and Green 2019). Precipitation during the spring and early summer of 2014 (88% of the 30 year average) was dramatically lower than that in 2015 and 2016 (156% and 133% of the 30 year average respectively; rainfall data from the Grand Junction, CO airport NWS site, approximately 21 km from the study area). We expected that food supplementation would increase survival, but this effect was only observed when the availability of natural food was limited. An unexpected result was that food supplementation negatively affected survival in 2015. These colonies suffered greater damage from rodents (prairie dogs and kangaroo rats) than did colonies transplanted in other years (92 of 135 supplemented colonies damaged). Large stores of seeds are attractive to rodents, and observations during activity censuses suggested that prairie dogs were most abundant in 2015.

Vegetation composition did not affect survival except in 2014, which had much less annual vegetation than 2015–2016. The significant interaction between the food treatment and vegetation PC2 in 2014 may have resulted from differences in locally available food resources that interacted with the food addition treatment, but it is difficult to be conclusive about an interaction that occurred only once. Plant species diversity measures potential food availability at the nest of these seed-harvesting ants, but foragers may travel up to 20 m from their nest in search of food. The scale of environmental variation that colonies experience was only partially captured by the vegetation score.

Survival varied at larger spatial scales, but not in any consistent way. Two of the sites (2015 and 2016) are contiguous and the other (2014) is within 300 m; the habitat in terms of perennial vegetation (shrubs and perennial grasses) is broadly similar. We did not measure all aspects of nest sites (e.g., slope, soil type, aspect, proximity to rodent burrows or to other ant species), instead relying on the large number of transplants and randomizing experimental treatments among locations. The differences in the spatial pattern of survival may have been influenced by one or more of these factors, in addition to the ones we manipulated directly. Although selection of habitat type by queens has been linked to colony survival in some ant species (e.g., King and Tschinkel 2016, Tschinkel and King 2017), this is based on large scale cues (e.g., areas of bare soil) rather than the fine scale cues that define individual nest sites in *P. occidentalis*. In our study species, habitat selection by queens that improves long-term colony success is extremely unlikely.

Differences in the spatial and temporal pattern of recruitment are commonly observed in other species that have a highly dispersive juvenile stage and a sedentary adult stage (e.g., plants: Wright et al. 2005; corals: Gleason 1996; barnacles: Caffey 1985, Jenkins et al. 2000). Temporal variation influences the characteristics that make a habitat favorable in a variety of plant species (Nicolè et al. 2011), and can influence the direction and pace of selection (Caruso and Peterson 2003). Variation among years can determine the magnitude of demographic impacts (Dahlgren and Bengtsson 2016, Tye et al. 2018), in some cases magnifying and in others ameliorating the effects.

In ants, the establishment of colonies by founding queens, and the growth and survival of young colonies, will determine the population dynamics of a species and its spatial distribution in the landscape (e.g., Vieira-Neto and Vasconcelos 2016). Although this stage of colony development is relatively unstudied, some patterns are consistent. Queens may respond to abiotic cues in selecting a nest site. Fire ant (*Solenopsis*) colonies have higher survival in disturbed areas (open soil). These clear sites have higher temperatures and high soil temperatures are important for brood development (Porter and Tschinkel 1993). Open or disturbed sites are also preferred by some species of *Atta* (Vieira-Neto and Vasconcelos 2010), perhaps because the higher temperatures of open sites facilitate the development of the fungus garden (Camargo et al. 2016). Queens of desert species (e.g., *Pogonomyrmex*, *Messor*), choose sites with soil characteristics that maximize their ability to get deep enough to avoid overheating (Johnson 1998, Motro and Motro 2016). Whether the queen is claustral (founds the colony without foraging as in *Solenopsis*, *Atta*, some species of *Pogonomyrmex*) or semiclaustral (continues to forage during colony founding as in *P. occidentalis*), will affect the sensitivity of the incipient colony to variation in the biotic environment (the availability of food and presence of predators). Studies on all species suggest that rapid growth by young colonies increases survival. Increasing worker number may enable colonies to increase food intake, increase efficiency (Clark and Fewell 2014), and resist brood raiding by con- or hetero-specifics (e.g., Fowler 1992, Tschinkel 1992).

Our study underscores the general importance of rapid growth and early attainment of large size in the survival of sessile species. Although annual variation that affects reproductive output may alter the number of propagules available for recruitment, factors which accelerate growth after settlement, germination or colony founding greatly increase the probability of juvenile

survival. Unlike many species of benthic invertebrates and reef fish (Marshall and Cook 2006), in which maternal investment enhances larval growth and survival, variation in propagule size had negligible effect beyond the initial stage of colony founding. Harvester ants, much like plants, have only a brief time window in the colony initiation period when parental investment makes a difference. Environmental quality, which can influence survival for both benthic invertebrates and plants, mattered less for ant colonies, except under extreme conditions. This difference between ants and other sessile species may be mediated by sociality. Ant colonies, which have multiple foragers that can acquire food at a distance from the nest site, may be less affected by site quality than other sessile species. The most distinctive difference between colony survival and juvenile survival in other sessile species was an attribute unique to ants. Colonies with multiply mated queens were more likely to survive over a wide range of circumstances, highlighting the importance of this trait even at the early stages of colony life.

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Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.3556/suppinfo

Open Research

Data (Cole and Wiernasz 2021) are available at Dryad: https://doi.org/10.5061/dryad.jm63xsj9v

Supporting Information: Cole, BJ, DM Jordan, M LaCour-Roy, S O'Fallon, L Manaker, JJ Ternest, M Askew, D Garey, DC Wiernasz. 2021. The benefits of being big and diverse: early colony survival in harvester ants. Ecology.

Appendix S1: Detailed description of methods

Colony construction. Reproduction in *P. occidentalis* is triggered by rainfall. The controlled mating technique uses experimental watering (8 - 10 L, using backpack sprayers) of specific colonies to induce the release of reproductives on the following day (Cole & Wiernasz 2000) which can be collected as unmated females and mated in specific ways. Females were collected by hand as they emerged from their nest; males were collected in traps, and carefully checked to remove any females. Eight to ten colonies served as queen sources on a given day, no males were used from these colonies. Males were divided evenly by volume among a number of 1.5 L containers equal to the number of female source colonies. Females were either paired with a single male selected arbitrarily from a different colony in a four dram vial, or placed in a 1.5 L plastic container with a large number (>300) of males representing all of the colonies that produced males on a given day. Singly paired females were given the opportunity to mate for at least 40 minutes while being continuously monitored, and were allowed to copulate until they separated naturally. Multiply-mated females were allowed to mate as many times as they wanted for 30-60 minutes before being removed from the containers. In 2015, early rainfall produced a reproductive flight before all colonies had been constructed. We augmented the number of females in the multiply mated category by collecting naturally mated queens while they were digging nest burrows. These queens are treated as multiply mated, and survived similarly to the experimentally mated queens. Because multiply mated queens in 2016 were a mix of experimentally mated females and collected foundresses, we performed a preliminary analysis of the survival of 2016 transplants to test whether the collection status of queens influenced survival. We included food supplement status, the size of the colony at transplant and the transplant set (see below) in the analysis and found no differences between the survival of queens collected after mating and those in controlled matings (Wilcoxon test, Chi-square = 0.50, $p = 0.48$).

Queen size was measured upon arrival at the University of Houston. In 2014, we used ImageJ to measure head width across the eyes to the nearest 0.1 mm from images of each queen taken with a Canon VIXIA HF11 camera. Size was the average of head width taken from two separate images. In 2015 and 2016, we measured queen wet mass to the nearest 0.001 mg using a Mettler AT20 analytical balance.

Transplant methods. We determined colony size (number of workers) before colonies were returned to the field to be transplanted. Eleven colonies were not counted. We shipped the juvenile colonies via overnight freight to Colorado in 20mm x 150mm test tubes partially filled with water (approximately 100 ants per tube, colonies consisted of 3-4 tubes). Upon arrival all ants from a single colony were transferred to a previously prepared 1 L cardboard container (15 cm tall, 12 cm upper diameter, 10 cm lower diameter, available from restaurant supply stores) with a tight fitting lid. Containers had been filled with local soil to about 3 cm from the top. We had added 75 ml of distilled water to each container, and had allowed two days for the water to fully penetrate the soil and equilibrate throughout the container before adding ants. The lid

remains on the container to minimize water loss. Containerized ants remained for two days in a cool location (~15-20°C), which allowed the colony to begin digging a nest within the soil of the tub.

Prior to transplanting experimental colonies, we had removed existing colonies of *P. occidentalis* by poisoning with hydramethylnon (Amdro®) which has a degradation half-life of 3.6 - 5.2 days in sunlight (Billick, et al 2001), identified transplant sites, and mapped these using a surveying theodolite (see Figure S1 for locations).

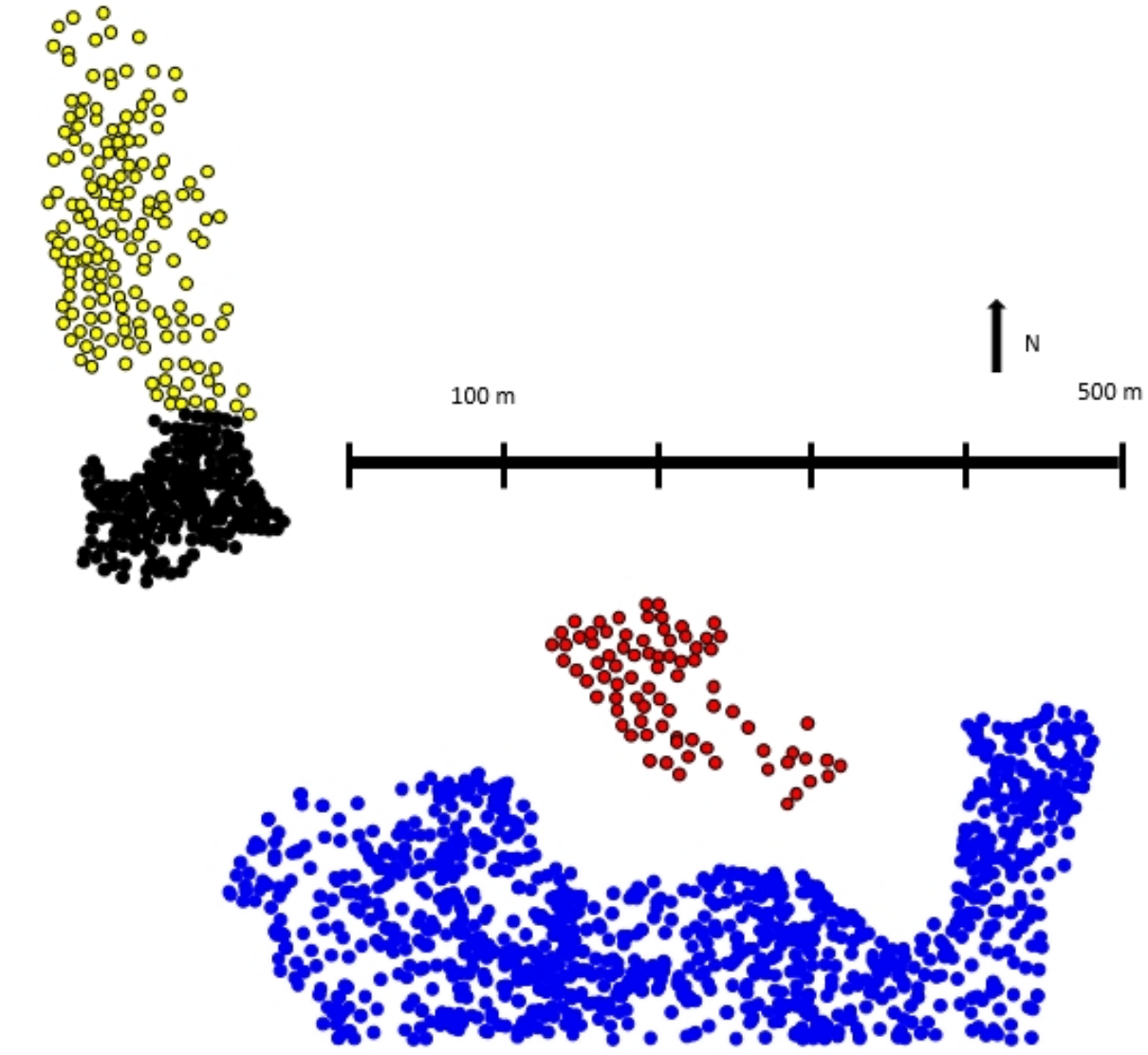

Figure S1. Map of the transplanted colonies. The location of 2014 transplants are in red, 2015 in yellow and the 2016 transplants are black symbols. The scale is in meters. The blue symbols represent the locations of nearby colonies in the long-term demographic study.

We transplanted 25-30 colonies at one time to avoid colony overheating; it usually took about 2 hours to transplant that number. Holes were dug with a garden trowel, and were made as narrow as possible while still fitting the container. The holes were dug so that about 3 cm of the tub would be above the ground, making the soil level at the nest site and in the container approximately equal. Using an extremely sharp utility knife, we cut a hole in the cardboard bottom of the tub, within 1 cm of the outside edge. This allowed the colony as large an area as possible for egress, while usually retaining the dirt within the tub. The space around the tub was then filled with dirt and a ring of aluminum flashing (15 cm high and about 22 cm in diameter; coated with liquid teflon) placed around the containers to protect against predatory species of ants (Figure S2). Lids were left on for one day, to encourage the ants to dig downward to establish the nest. The following day we removed the lids and provided 2 g of mixed seeds. The ring remained for two days to provide additional protection while the colony was being established. When the ring was removed, we cut the rim of cardboard from the container so that it was level with the ground (Figure S2). In 2014, all transplanting was done in the evening (1800 - 2100 hrs) to avoid transplanting during the hottest period of the day. In 2015 and 2016, cool, rainy weather enabled us to transplant in the morning (0700 - 1200 hrs). We transplanted as many as 75 colonies in one day.

Figure S2. Transplanted colonies. A. A newly transplanted colony showing the aluminum ring placed around the cardboard tub that contains the transplanted colony. This ring is to protect colonies from intrusions by ants from other colonies. Note the fresh soil on the surface of the transplanted nest. B. A transplanted colony after the ring has been removed. In both figures worker ants are visible on the surface.

Monitoring Survival. Although we transplanted a total of 584 colonies, 10 colonies were never active after transplanting, and were assumed to have died during the process. Colonies were observed 2-3 meters from the nest, using binoculars, until either workers were seen entering or leaving the nest or until two minutes had elapsed. In 2014, a single observer (DJ) was sufficient to monitor all colonies, but the larger transplant cohorts of 2015 (ML, SO, LM) and 2016 (JT, MA, DG) required multiple observers. The order of colony observation was varied daily to account for differences in the timing of activity. In 2015 and 2016, each observer monitored approximately 1/3 of the colonies for two consecutive days, and then switched, so that each person monitored each colony twice per week. In all years BJC and DCW monitored colonies in August-September.

Vegetation measurements. In 2014 we measured the percent cover for each species, including the amount of bare ground and microbiotic soil in a 1m radius around each nest. In 2015-2016 we assayed vegetation at a nest site by placing a 1x1 m quadrat arbitrarily 1 m north of the transplanted nest. The quadrat was divided into 0.25 m^2 squares and the number of times that a species was represented in quarter meter subplots was recorded (0-4). The principal components of the vegetation were extracted using the covariance matrix and we calculated the factor score for each transplanted colony. We extracted the principal components using common plant species (all species with an average presence of 1 or an average of greater than 1% cover), microbiotic soil and bare ground. Because the values for bare ground and microbiotic soil were on such different scales from the plant cover in 2014 we used the correlation matrix to calculate principal components.

Statistical Methods and Analysis.

Survival analysis. The Cox proportional hazards model predicts the chance of dying through time, termed the hazard, as a function of multiple treatments. The model to be fitted is:

$H(t) = H_0(t) \times e^{\sum_{i=1}^{p} \beta_i X_i}$, where $H_0(t)$ is the baseline hazard, the X_i are the p experimental treatments, and the β_i are the coefficients that measure the strength of the treatments and are estimated using maximum likelihood. For all colonies, the chance that death occurs is a function of time, but the probability of mortality is influenced by the p experimental treatments (the X_i). If a treatment increases the probability of mortality, then the β will be larger. Treatment effects were compared using a hazard ratio, defined as the hazard of not having a hypothesized beneficial treatment, such as increased food or multiple mating, divided by the hazard of having the beneficial treatment. For a single factor the expression for the hazard ratio simplifies to: $HR = \exp[\beta \cdot (X^* - X)]$, where the X* is the hypothesized reference treatment (e.g. no additional food) and X is the hypothesized positive treatment (food supplement). Because the standard is to code the reference value as 1 and the positive treatment as 0, the hazard ratio for a single treatment is just exp[β].

Vegetation analyses. For each year, we used the main effects of food, mating category, size at transplant and the first two vegetation component scores as covariates in a proportional hazards model. We also used the mate x food and mate x size interactions because these were relevant in the overall survival analysis, as well as the interactions between the vegetation scores and the food treatment. Our reasoning was that vegetation may have an independent effect on survival,

and if it interacted with other variables, then an interaction with food addition would be the most interpretable. For the 2015-2016 survival data we stratified the analyses by transplant set.
Queen size analyses. We included queen size as a covariate in a survival analysis that used the main effects, food, mating and colony size at transplant as well as the interaction effects mate x food and mate x size as in the vegetation analyses.
Spatial pattern of mortality. For each colony that was dead at the end of the summer, we calculated the mean distance to its 6 nearest dead neighbors. For each year the observed values of these distances were compared to random expectations. To compute the random expected values we used the same colony locations but randomized the assignment of mortality across colonies. For each iteration we calculated the mean distance to the 1st-6th nearest dead neighbors. We performed 1000 iterations and calculated the neighborhood spectrum of the expected distance to the 1st-6th nearest colonies as well as the standard deviation of the mean distances (= standard error). We calculated: (Observed - Expected)/(standard deviation of expected value). This is a standard normal deviate which can be tested with a z-test.

References

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Supporting Information: Cole, BJ, DM Jordan, M LaCour-Roy, S O'Fallon, L Manaker, JJ Ternest, M Askew, D Garey, DC Wiernasz. 2021. The benefits of being big and diverse: early colony survival in harvester ants. Ecology.

Appendix S2: Detailed results for the effects of maternal lineage and queen size.

Table S1. Effects of maternal lineage. The full survival analysis (before any variables are removed). Maternal lineage is the first variable that would be removed in a stepwise analysis.

	Joint Tests		
Effect	DF	Wald Chi-Square	Probability
mate	1	5.024	0.025
food	1	0.083	0.772
size_transp	1	5.172	0.023
lineage	39	23.554	0.976
mate*food	1	3.188	0.074
size_transp*mate	1	5.385	0.020
size_transp*food	1	0.340	0.560
size_trans*mate*food	1	2.066	0.151
food*set(year)	4	11.605	0.021
mate*set(year)	4	9.720	0.045
size_trans*set(year)	4	19.095	0.001

Table S2. Effects of queen size. The initial survival analyses with queen size as a covariate in the analysis. Queen size was not significant in any initial model. Queen size was not retained as significant in any stepwise analysis (it is the first variable removed in 2016, the second variable removed in 2015, and the last variable removed in 2014).

2014

	Joint Tests		
Effect	df	Wald Chi-Square	Pr > ChiSq
size_transp	1	2.8704	0.0902
food	1	0.0321	0.8579
mate	1	0.8334	0.3613
queensize	1	2.6403	0.1042
mate*food	1	0.2948	0.5872
size_transp*mate	1	0.9580	0.3277
size_transp*food	1	0.0409	0.8396

2015

Joint Tests

Effect	df	Wald Chi-Square	Pr > ChiSq
size_transp	1	3.4884	0.0618
food	1	1.1265	0.2885
mate	1	0.1378	0.7105
queensize	1	0.3991	0.5276
mate*food	1	0.6389	0.4241
size_transp*mate	1	0.0102	0.9194
size_transp*food	1	2.7401	0.0979

2016

Joint Tests

Effect	df	Wald Chi-Square	Pr > ChiSq
size_transp	1	2.4712	0.1159
food	1	5.9142	0.0150
mate	1	4.6153	0.0317
queensize	1	0.0228	0.8801
mate*food	1	3.4251	0.0642
size_transp*mate	1	3.8934	0.0485
size_transp*food	1	5.6107	0.0179

Supporting Information: Cole, BJ, DM Jordan, M LaCour-Roy, S O'Fallon, L Manaker, JJ Ternest, M Askew, D Garey, DC Wiernasz. 2021. The benefits of being big and diverse: early colony survival in harvester ants. Ecology.

Appendix S3: Detailed results for the effect of vegetation composition.

For each year we present the results of the principal components analysis of the vegetation. We present the results of survival analysis including the first two principal components scores in the analysis. In the analyses we present the starting and final model for a stepwise survival analysis. In 2014 the vegfactor2*food interaction was significant in the initial model and remained significant after the backwards stepping procedure. In 2015 and 2016 neither the of vegetation scores nor their interactions with food were significant either in the starting or final model in the stepwise analysis.

Vegetation analysis for 2014

Principal Component Loadings

	1	2
Bare ground	-0.93516	0.15615
Microbiotic soil	-0.34687	-0.04361
Hilaria jamesii	0.68715	-0.54694
Atriplex gardneri	0.34371	0.15705
Gutierrezia sarothrae	0.09179	0.81374
Tetradymia spinosa	0.54572	0.40112
Sphaelecera coccinea	-0.39025	-0.43268
Atriplex confertifolia	0.17085	-0.05672
Percent of Total Variance Explained	25.91	17.04

Initial model: Size at transplant, food, mate, vegfactor1, vegfactor2, vegfactor1*food, vegfactor2*food, mate*food, Size at transplant*mate, Size at transplant*food, Size at transplant*mate*food

Final model:

Joint Tests

Effect	df	Wald Chi-Square	P
Size at Transplant	1	8.67	0.003
Food	1	0.29	0.59
Mate	1	2.57	0.11
Vegfactor2	1	2.50	0.11
Vegfactor2*food	1	6.28	0.012
Size at Transplant*mate	1	2.96	0.085

Vegetation analysis for 2015

Principal Component Loadings

	1	2
Hilaria jamesii	-1.25058	0.43394
Bromus tectorum	0.39539	1.39948
Atriplex gardneri	1.03087	-0.00249
Gutierrezia sarothrae	-0.33550	-0.20955
Malcolmia, africanus	0.14233	-0.18711
Xylorhiza venusta	0.15629	-0.28202
Erigeron pulcherrimus	0.15932	-0.04928
Oenothera caespitosa	-0.03740	-0.03720
Tetradymia spinosa	0.00034	0.04358
Picrothamnus desertorum	0.04903	-0.00133

Sphaelecera coccinea	0.02071	0.00019
Limus ambiguous	0.03004	0.00564
Ephedra torreyana	-0.02124	0.00326
Chrysothamnus viscidiflorus	-0.03752	-0.01040
Atriplex confertifolia	0.00333	0.04070
Nostoc commune	0.03485	0.01208
Syntrichia canineruis	-0.02638	0.08478
Percent of Total Variance Explained	25.28	19.95

Initial model: Size at transplant, food, mate, vegfactor1, vegfactor2, vegfactor1*food, vegfactor2*food, mate*food, Size at transplant*mate, Size at transplant*food, Size at transplant*mate*food, Size at transplant*mate*set, Size at transplant*food*set, Size at transplant*mate*food*set

Final model:

Joint Tests

Effect	df	Wald Chi-Square	P
Size at Transplant	1	4.42	0.036
Food	1	3.08	0.079
Mate	1	3.40	0.065
Size at Transplant*set	1	2.52	0.11
Size at Transplant*mate*set	1	4.92	0.027

Vegetation analysis for 2016

Principal Component Loadings

	1	2
Hilaria jamesii	0.18346	-0.75407
Bromus tectorum	0.49439	-0.03593
Atriplex gardneri	-0.58703	0.05790
Gutierrezia sarothrae	0.27017	0.09418
Malcolmia, africanus	0.56494	0.43895
Xylorhiza venusta	-0.61037	-0.10707
Erigeron pulcherrimus	-0.24871	-0.49350
Tetradymia spinosa	-0.02642	0.24575
Sphaelecera coccinea	0.18803	-0.02850
Atriplex confertifolia	0.04032	0.45204
Hordeum jubatum	-0.50765	0.50365
Alyssum minus	0.50059	0.06683
Atriplex corrugata	-0.30555	0.18186
Percent of Total Variance Explained	16.07	12.20

Initial model: Size at transplant, food, mate, vegfactor1, vegfactor2, vegfactor1*food, vegfactor2*food, mate*food, Size at transplant*mate, Size at transplant*food, Size at transplant*mate*food, Size at transplant*mate*set, Size at transplant*food*set, Size at transplant*mate*food*set

Final model:
vegfactor1: df=1, chi-square=2.71, p=0.10

Supporting Information: Cole, BJ, DM Jordan, M LaCour-Roy, S O'Fallon, L Manaker, JJ Ternest, M Askew, D Garey, DC Wiernasz. 2021. The benefits of being big and diverse: early colony survival in harvester ants. Ecology.

Appendix S4: Detailed results for the spatial pattern of mortality.

Table S1. Spatial analysis of transplant survival. The observed 1st - 6th average distance (m) to the nearest neighbors among colonies dead at the end of the transplant year. The expected values are the average 1st - 6th distance to nearest neighbors when colonies are randomized by mortality. The SE for a year is the standard deviation of the randomized differences and the z for a given year is (Obs-Exp)/SE. The significance is * $p < 0.05$, ** $p < 0.01$

Nth Nearest neighbor	2014 Observed	2014 Expected	SE 2014	z2014	2015 Observed	2015 Expected	SE 2015	z 2015	2016 Observed	2016 Expected	SE 2016	z 2016
1	10.32	9.69	0.49	-1.31	8.74	8.31	.32	1.34	6.76	9.84	0.94	-3.28**
2	13.57	13.26	0.70	-0.45	11.4	11.5	.40	-.25	11.2	14.5	1.19	-2.77**
3	17.47	16.71	0.81	-0.94	13.7	14.3	.46	-1.30	15.0	18.3	1.42	-2.32*
4	20.20	19.78	0.93	-0.45	15.6	16.7	.50	-2.20*	19.7	21.5	1.63	-1.10
5	22.75	22.74	1.12	-0.01	17.4	18.9	.54	-2.78**	22.8	24.4	1.84	-0.87
6	25.90	25.75	1.33	-0.11	19	20.9	.60	-3.17**	25.0	27.2	2.04	-1.08

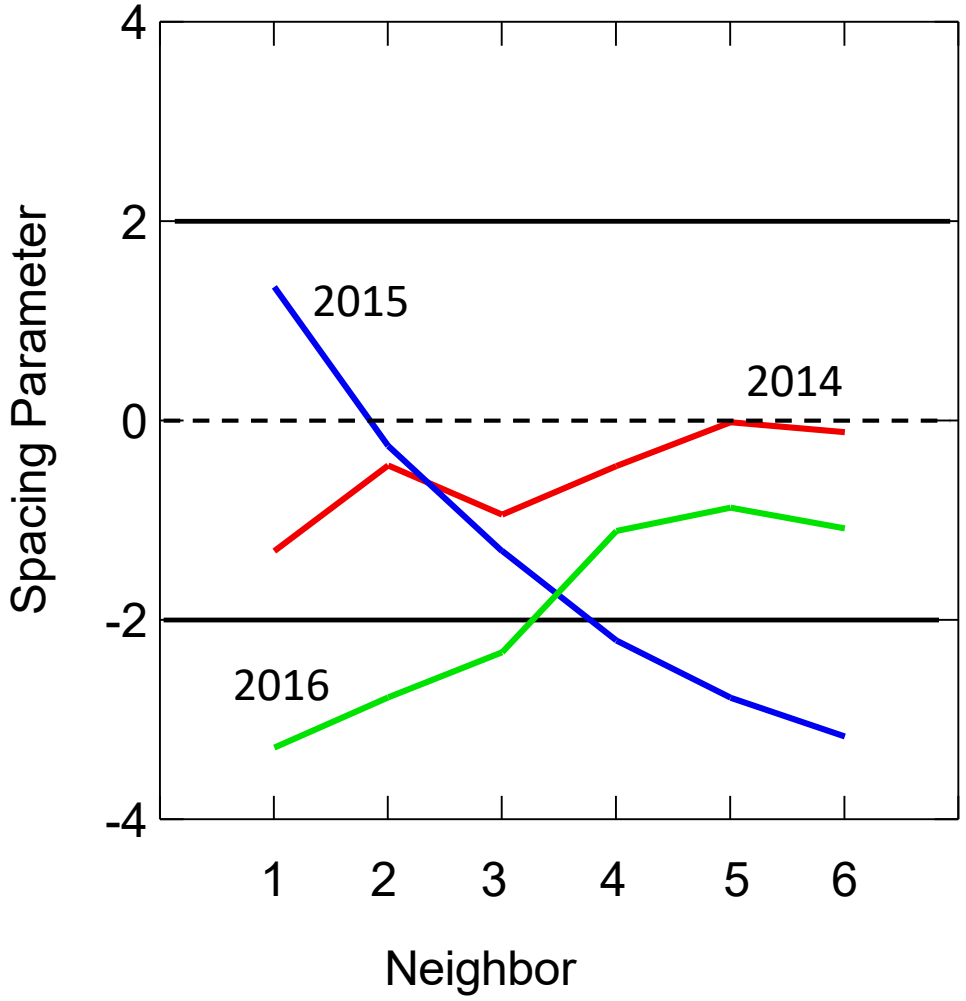

Figure S1. The z-scores for the spatial analysis shown graphically. The expected z-scores is zero (dashed line) with ± 2 shown as 95% confidence intervals (solid lines).