



Original Article

Harvester ant nest architecture is more strongly affected by intrinsic than extrinsic factors

Sean O’Fallon,^{a,*} Eva Sofia Horna Lowell,^{a,*} Doug Daniels,^b and Noa Pinter-Wollman^{a,●}

^aDepartment of Ecology and Evolutionary Biology, University of California, Los Angeles, 610 Charles E Young Dr S, Los Angeles, CA 90095, USA, and ^bData Science Center, UCLA Library, 280 Charles E Young Dr N, Los Angeles, CA 90095, USA

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Behavior is shaped by genes, environment, and evolutionary history in different ways. Nest architecture is an extended phenotype that results from the interaction between the behavior of animals and their environment. Nests built by ants are extended phenotypes that differ in structure among species and among colonies within a species, but the source of these differences remains an open question. To investigate the impact of colony identity (genetics), evolutionary history (species), and the environment on nest architecture, we compared how two species of harvester ants, *Pogonomyrmex californicus* and *Veromessor andrei*, construct their nests under different environmental conditions. For each species, we allowed workers from four colonies to excavate nests in environments that differed in temperature and humidity for seven days. We then created casts of each nest to compare nest structures among colonies, between species, and across environmental conditions. We found differences in nest structure among colonies of the same species and between species. Interestingly, however, environmental conditions did not have a strong influence on nest structure in either species. Our results suggest that extended phenotypes are shaped more strongly by internal factors, such as genes and evolutionary history, and are less plastic in response to the abiotic environment, like many physical and physiological phenotypes.

Key words: collective behavior, extended phenotypes, nest architecture, social insects.

INTRODUCTION

Within and across species, phenotypic variation plays an important role in evolution (Lewontin 1974; Mayr 2013). Both intrinsic and extrinsic factors contribute to phenotypic variation (West-Eberhard 2003; Roff 2012), with differing implications for natural selection. Within a species, individuals have different phenotypes due to many intrinsic differences (McEntire et al. 2021) such as their genotype (Falconer and Mackay 1996; Lynch and Walsh 1998), parental effects (Danchin et al 2011; Lehto and Tinghitella 2020), etc. Differences in traits among species can reflect different evolutionary histories (Blount et al. 2018) and can shape community composition (Hochwender and Fritz 2004) and evolvability of traits (Kokko et al. 2017). Extrinsic factors, such as environmental conditions, also contribute to phenotypic differences. Temperature (Atkinson 1994; Patel and Franklin 2009; Singh et al. 2020) and humidity (Lorenzon et al. 2001) can affect the development of physical and

behavioral traits. Due to the importance of variation to ecological and evolutionary patterns and processes, the relative contributions of intrinsic and extrinsic factors to variation in phenotypes have been the subject of intense research (Warner and Andrews 2002; Fischer et al. 2004).

Like traditional phenotypes, extended phenotypes differ among individuals (Lissåker and Kvarnemo 2006; Rushbrook et al. 2008; Järvinen and Brommer 2020) and species (Collias 1997; Stone and Cook 1998). Extended phenotypes are traits expressed outside the body of an organism, such as the effects of behavior on the physical environment (Dawkins 2016). Like other traits and behaviors, individuals can consistently differ in their extended phenotypes. Indeed, in social insects, in which a colony is the unit on which selection acts (Hölldobler and Wilson 2009), there are consistent individual differences among colonies in nest structure (Mizumoto and Matsuura 2013; DiRienzo and Dornhaus 2017), suggesting that these differences may be driven by intrinsic factors. Across species, genetic differences can lead to species-specific extended phenotypic traits (Weber et al. 2013). Extended phenotypes are not only shaped by natural selection (Dawkins 2016) but can themselves

*Equal contributors.

Address correspondence to S. O’Fallon. E-mail: seanofallon@ucla.edu.

exert selective pressures on related traits through niche construction (Odling-Smee et al. 1996). Furthermore, factors that are extrinsic to an individual, like climatic environmental conditions, can contribute to variation in extended phenotypes because individuals may respond to the environment when expressing their extended phenotype (Blamires 2010). Furthermore, extrinsic factors can contribute to interspecific differences in extended phenotypes across species that experience different environmental conditions (Biddle et al. 2018). Understanding how extended phenotypes shape and are shaped by natural selection can be achieved by disentangling the contributions of intrinsic and extrinsic factors to their structure (DiRienzo and Aonuma 2018).

Nests are extended phenotypes with structures that differ among individuals and species (Hansell 2007). Ants construct and excavate a wide range of nests with a diversity of structures across and within species (Tschinkel 2021). Therefore, ants are an ideal study system for examining the factors that determine variation in extended phenotypes (Kleineidam and Roces 2000; Penick and Tschinkel 2008). Within ant species, nests differ among colonies in structural features such as number of chambers (Tschinkel 2004; Tschinkel 2005; Verza et al. 2007; Guimarães et al. 2018), total depth (Kleineidam and Roces 2000; Tschinkel 2004; Tschinkel 2005; Guimarães et al. 2018), and connectivity of chambers (Pinter-Wollman 2015). Nest structure also differs across species in their depth and in the number, size, spacing, and connectedness of tunnels and chambers (Sudd 1970; Tschinkel 2011; Tschinkel 2015). Differences in extrinsic factors, such as environmental conditions, can impact the structure of nests that colonies construct. Soil temperature (Bollazzi et al. 2008) and humidity (Pielström and Roces 2014) affect the depth of nests; CO₂ concentrations affect chamber depth (Römer et al. 2017) and the presence of ventilating turrets (Halboth and Roces 2017); and atmospheric temperature and rainfall affect the length and slope of nest mounds (Vogt et al. 2008). Through these responses to extrinsic factors, ant colonies can buffer the external environmental conditions in which they live (MacKay 1981; Kleineidam and Roces 2000; Penick and Tschinkel 2008).

Harvester ant species differ considerably in colony sizes and collective foraging behavior (Johnson 2000), and so their intrinsic traits may place different demands on the size and structure of their nests. Here we ask what factors influence extended phenotypes, that is, nest structure, by comparing nests excavated by workers from two harvester ant species from separate tribes of the Myrmicinae subfamily (Ward et al. 2015), *Pogonomyrmex californicus* and *Veromessor andrei*. *Veromessor andrei* colonies form foraging columns (Johnson 2000) and regularly relocate nest sites (Brown 1999; Pinter-Wollman and Brown 2015), whereas *P. californicus* forage individually (Johnson 2000) and relocate to a new nest site only rarely (De Vita 1979). Because *V. andrei* colonies exhibit collective behavior that relies on nestmate recruitment more than *P. californicus* colonies, we predict that *V. andrei* nests will contain wider, more connected tunnels that facilitate interactions and rapid recruitment. Furthermore, *V. andrei* has larger body lengths than *P. californicus*, but they do not differ in head width (Fig. S1). These differences in morphology could impact the size of the tunnels each species dig because larger ants remove larger amounts of dirt while digging (Gravish et al. 2012; Kwapich et al. 2018).

Different extrinsic factors may influence the extended phenotypes of the two ant species we study. Although the geographical ranges of *P. californicus* and *V. andrei* overlap in southern California (Janicki et al. 2016; Guénard et al. 2017), the two species occupy different habitats and experience different temperature and

humidity ranges. *Pogonomyrmex californicus* colonies occur in drier, hotter conditions than colonies of *V. andrei*, which may result in higher levels of desiccation risk for *P. californicus* than for *V. andrei* as in other *Pogonomyrmex* (Lighton and Feener 1989) and *Veromessor* (Feener and Lighton 1991) species. Thus, it is possible that the structure of *P. californicus* nests will be better adjusted to hot and dry environments, with smaller chambers and fewer tunnel connections compared with *V. andrei* nests.

We investigate the relative contribution of intrinsic factors, specifically evolutionary history, and genotype and extrinsic factors, specifically temperature and humidity, to an extended phenotype—the structure of subterranean nests of harvester ants. To examine the contribution of intrinsic factors, we compare the structure of nests constructed by different colonies of two species of harvester ants. To determine the effect of extrinsic factors, we compare nest structures excavated in different temperatures and humidities.

METHODS

Animal collection and maintenance

We collected and brought into the lab harvester ant workers from four different colonies from each of the two species *P. californicus* and *V. andrei* ($N = 8$ colonies). *Pogonomyrmex californicus* workers were collected from colonies along the Red Rock Road in Red Rock Canyon Park in the Santa Monica Mountains. *Veromessor andrei* workers were collected from colonies on the southwest mesa at the UC Sedgwick Reserve in the Santa Ynez Valley. For each species, we identified the four most active colonies in the population to ensure that we could collect sufficient ants for all the experimental trials. We recorded the location of each colony so that we could return to them throughout the study. We collected ants multiple times throughout the eight weeks of the study (June to August 2020) and began the experiments 48 h after each collection so that ants were housed in the lab for no more than 48 h before beginning digging trials. To collect the ants, we wore latex gloves and placed 100–120 workers in a plastic container that had a wet paper towel for moisture.

In the lab, we placed ants from each of the eight colonies in a separate plastic container with a lid and flouon along the walls to prevent ants from escaping. We supplied the workers with ad libitum water and 50% sugar water in glass tubes plugged with cotton to allow wicking of the fluid during the 48 h before the digging trials began and the 7 days of the trial itself.

Experimental design

We allowed 50 workers from each of the eight source colonies to excavate a nest in one of four environmental treatments (Figure 1): cold/dry, cold/wet, hot/dry, hot/wet. To control for the potential impact of the number of workers on nest size, we used groups of identical size (50 workers) in each experiment. Temperature and humidity were controlled using environmental chambers (Caron, model 6045) with “hot” temperature set at 29.4 °C and “cold” at 19.5 °C. The air temperature set in the environmental chamber was very similar to that inside the soil buckets (0.54 ± 1.48 °C difference; see Supplementary Materials, Table S4). The “wet” condition was 80% humidity and “dry” was on average 23% humidity (range: 17–29%). The “dry” condition had some variability because of the technological limits of the environmental chambers. The two experimental temperature values and two experimental humidity values we tested represent the range of typical daytime conditions

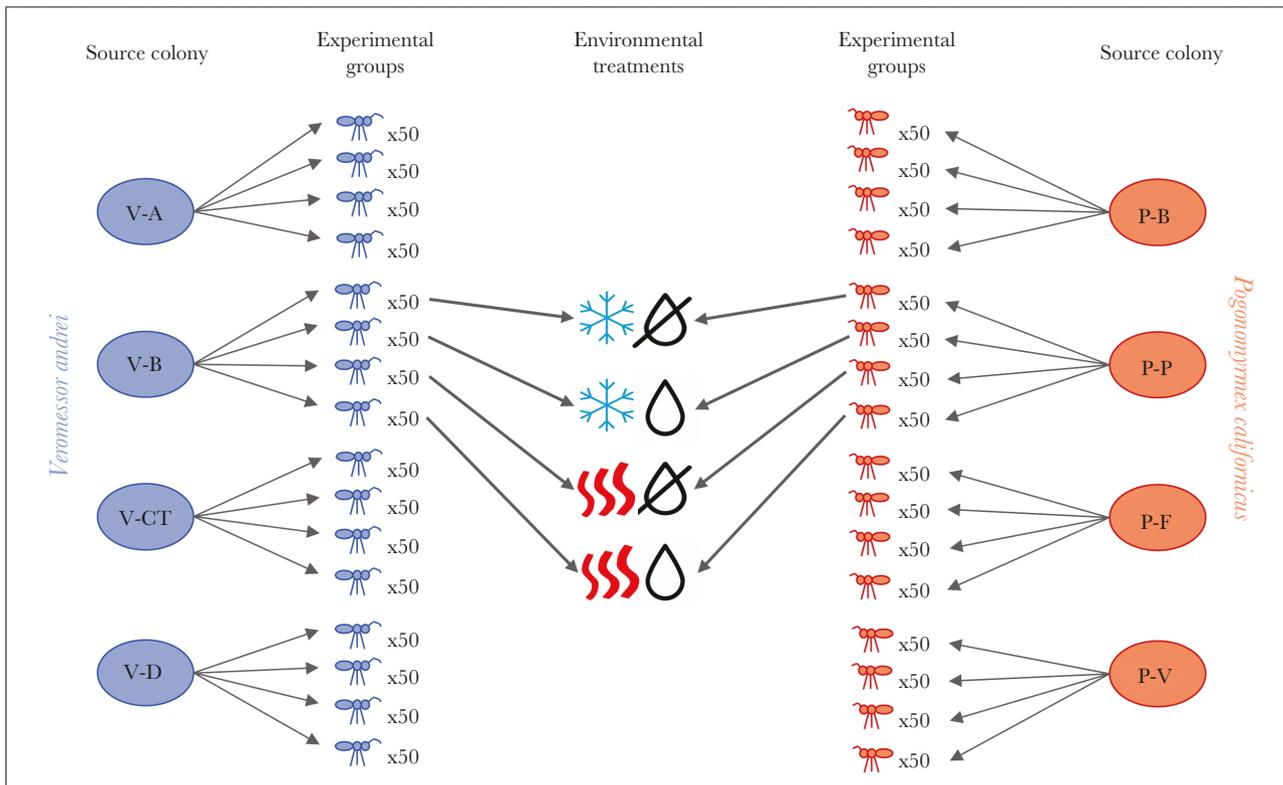


Figure 1

Schematic of the experimental design. We studied two species of harvester ants: *V. andrei* (blue), and *P. californicus* (orange). We formed four experimental groups of 50 ants from each of eight source colonies—four source colonies from each species. The source colonies are depicted as ovals with letters indicating colony ID. Colony ID is comprised of the first letter of the species name (V/P) followed by the first letter of the colony name as it appears in the figures below and in the data (O'Fallon et al. 2022). Each of the four experimental groups from each source colony was placed in a nest excavation trial under Cold/Dry, Cold/Wet, Hot/Dry, or Hot/Wet environmental conditions. Cold (snowflake symbol) was 19.5 °C, hot (red curved lines) was 29.4 °C, dry (drop symbol with a line across) was 23% humidity, and wet (drop symbol) was 80% humidity. Arrows show this assignment to treatment for only eight experimental groups to maintain figure clarity, but all experimental groups were assigned to the environmental treatments in a similar manner.

that the populations we collected ants from experience throughout the year (according to wunderground.com and Western Regional Climate Center, see [Supplementary Materials](#)). The “cold” treatment represents average daytime highs during the coldest months (December–March) and the “hot” treatment represents average daytime highs during the warmest months (July–October) at both field sites ([Table S2](#)). The “wet” treatment represents the highest average humidity recorded at the two sites ([Table S3](#)) and the “dry” treatment humidity was the driest conditions we could achieve in the environmental chambers.

Nest excavation trials

To allow ants to dig a nest, we placed the 50 workers in a five-gallon bucket filled with 47 cm of Quikrete All-Purpose sand moistened with approximately 150 mL water. We placed the maximum amount of sand possible in the buckets (47 cm) without having ants escape. We used store-bought sand to provide all ant groups with controlled identical digging conditions. In preliminary trials, we found that the amount of sand we used needed to be moistened with approximately 150 mL water to allow the ants to dig stable nest structures that can be casted and excavated. To focus the digging of the ants at one location, and prevent them from digging multiple nest entrances, we covered the sand with a layer of hardened wax with one circular opening (7–10 mm diameter) in the

center of the wax cover ([Figure S2](#)). This circular opening acted as the nest entrance. Throughout the nest excavation trials, we supplied the ants with ad libitum water and 50% sugar water in glass tubes plugged with cotton to allow wicking of the fluid. Ants were allowed to excavate a nest for seven days with a 12 h day/night light regime. This experimental set up is similar to the one used in other studies of harvester ant nest excavation ([Kwapich et al. 2018](#)). To create a cast of the excavated nest, at the end of the week of excavation, we evacuated workers from the nest by blowing air into the nest entrance. When most ants were evacuated, we poured melted wax down the nest entrance opening and after the wax hardened, we carefully dug out the cast for further quantification ([Figure S2](#)).

Cast quantification

To quantify the nest structures that the ants excavated we measured features of the tunnels, chambers, and of the entire nest, as described below. We then compared these features among colonies and between species, temperature, and humidity treatments.

Tunnel measurements

Tunnels are an important feature of the nest because they facilitate movement of ants and materials. To determine the capacity of each tunnel to transport material and ants we measured the length and circumference of each tunnel segment. We defined a tunnel

segment as a tunnel connecting two nest features (chambers, junctions, nest entrance, or ends of cast). We laid a string along the tunnel segment and measured its length with a ruler to the nearest mm. To measure the circumference of each tunnel segment, we wrapped a string around the tunnel segment at each of its terminals and at its center, and then recorded the length of the string with a ruler to the nearest mm. We recorded the average of these three measurements as the tunnel circumference.

Chamber measurements

A chamber was defined as any section of the nest with a globular, rather than cylindrical, shape. To quantify the capacity of each chamber to house ants and other materials, we measured chamber circumference by wrapping a string around the chamber's widest point and recording the length of the string with a ruler to the nearest mm.

Global nest measurements

To quantify the global structure of each nest we used network analysis to quantify nest shape, combined nest-level tunnel and chamber measurements, and measured nest volume. All measures were aggregated using a PCA, as detailed below. We depicted each nest as a network of nodes representing chambers, junctions, and ends of the cast, connected by edges representing tunnels, as in (Buhl et al. 2004; Perna et al. 2008; Viana et al. 2013; Gautrais et al. 2014; Pinter-Wollman 2015) (see Supplementary Materials for examples). To characterize nest connectivity, which can impact a colony's speed of recruitment to food (Pinter-Wollman 2015), we computed the average degree, which is the mean number of unique nodes that each node is connected to. To determine how well different nest elements are connected with one another, we calculated network density, which is the number of observed edges divided by the number of possible edges. To determine the distances between different nest elements, which can impact how quickly resources and information flow through the nest, we calculated average path length, which is the mean number of edges that connect all node pairs. To further quantify the potential for flow through the nest, we noted the presence or absence of cycles. A cycle is defined as a path along a network that returns to its starting node after passing through at least one other node (Bollobás 1998; Gross and Yellen 2005; Bender and Williamson 2010; Balakrishnan 2011; Newman 2018). Larger colonies have more cycles (Buhl et al. 2004) and colonies in nests with more cycles have faster recruitment to food (Pinter-Wollman 2015).

We further calculated measures of nest size: total number of chambers, average circumference of all tunnel segments, total length of all tunnel segments, and nest volume. To obtain volumes we scanned each nest fragment individually using a professional structured light scanner (Artec Space Spider) with an accuracy of 100–200 microns. To help the scanner detect the otherwise semi-translucent wax, we painted exposed sections of the wax in dark blue. We then manually aligned each digitized fragment with other fragments from the same nest using Artec Studio. Once each nest was aligned, we loaded models into Meshmixer to turn the disparate fragments into a single STL file. We imported the STL files into Meshlab, which provides volumetric measurement under the Mesh option.

Data analysis

To aggregate the various global nest measures we used a Principal Component Analysis (PCA). In the PCA we included all measures

listed in the “Global nest measurements” section above, except for the presence or absence of cycles, which is a binary variable and was thus analyzed separately. Each measure was scaled by subtracting its mean and dividing by its standard deviation before running the PCA. The first principal component explained 61.9% of the variance (Table S2) therefore we used it as a single measure of “nest size” (Table S2, Figure S3).

To determine the effect of species, colonies, and environmental conditions on the excavated nest tunnels, chambers, and global nest features, we conducted four separate ANOVAs and one logistic regression (for cycles in the nest). In each statistical test, the measures of tunnels (circumference or length), chambers (circumference), or global nest (nest size or presence/absence of cycles) were the dependent variables. We log-transformed the tunnel measures to meet the ANOVA assumptions; chambers and nest size measures met the ANOVA assumptions. All statistical models included species, colony ID nested within species, temperature, and humidity as independent variables. All statistical analysis was conducted in R version 4.0.3 (R Core Team 2020). The ANOVAs were conducted using the `Anova()` function from the package “car” (Fox and Weisberg 2018). Data and analysis code are available in the Supplementary Materials.

RESULTS

Impact of species and colony identity on nest architecture

Both intrinsic factors, species and colony identity, had a significant effect on tunnel and global nest measures but not on chamber size. Both species and colony identity had a significant impact on tunnel circumference (Figure 2A,B). *Veromessor andrei* dug nests with wider tunnels compared with *P. californicus* (ANOVA: $F = 31.07$, $df = 1$, $P < 0.0001$, Figure 2A, Table 1) and ants from different source colonies dug nests with different tunnel circumferences (ANOVA: $F = 5.81$, $df = 6$, $P < 0.0001$, Figure 2B, Table 1). Colony identity, but not species, significantly impacted the number of nests with cycles and nest size. Colonies differed in the number of nests with cycles (Logistic regression: $\text{Chisq} = 16.64$, $df = 6$, $P = 0.011$, Figure 3B, Table 2) but species did not (Logistic regression: $\text{Chisq} = 0.474$, $df = 1$, $P = 0.491$, Figure 3A, Table 2). Similarly, colonies differed in their nest size (ANOVA: $F = 2.92$, $df = 6$, $P = 0.031$, Figure 3D, Table 1) but we did not detect differences between species (ANOVA: species: $F = 0.015$, $df = 1$, $P = 0.904$, Figure 3C, Table 1). We did not detect an effect of either intrinsic factor, species or colony identity, on tunnel length (ANOVA: species: $F = 0.628$, $df = 1$, $P = 0.429$, Figure 2C; colony: $F = 1.523$, $df = 6$, $P = 0.169$, Figure 2D, Table 1), or chamber circumference (ANOVA: species: $F = 2.224$, $df = 1$, $P = 0.146$, Figure 2E; Colony ID: $F = 0.177$, $df = 6$, $P = 0.981$, Figure 2F, Table 1).

Impact of temperature and humidity on nest architecture

The only feature that was influenced by the extrinsic variables that we tested was the number of nests with cycles. We did not detect a significant effect of temperature on tunnel circumference, tunnel length, or chamber circumference (ANOVA: tunnel circumference: $F = 0.011$, $df = 1$, $P = 0.918$, Figure 4A, Table 1; tunnel length: $F = 0.091$, $df = 1$, $P = 0.763$ Figure 4C, Table 1; chamber circumference: $F = 0.008$, $df = 1$, $P = 0.930$, Figure 4E, Table 1). Similarly, we did not find a significant effect of humidity on tunnel circumference, tunnel length, or chamber circumference (ANOVA: tunnel

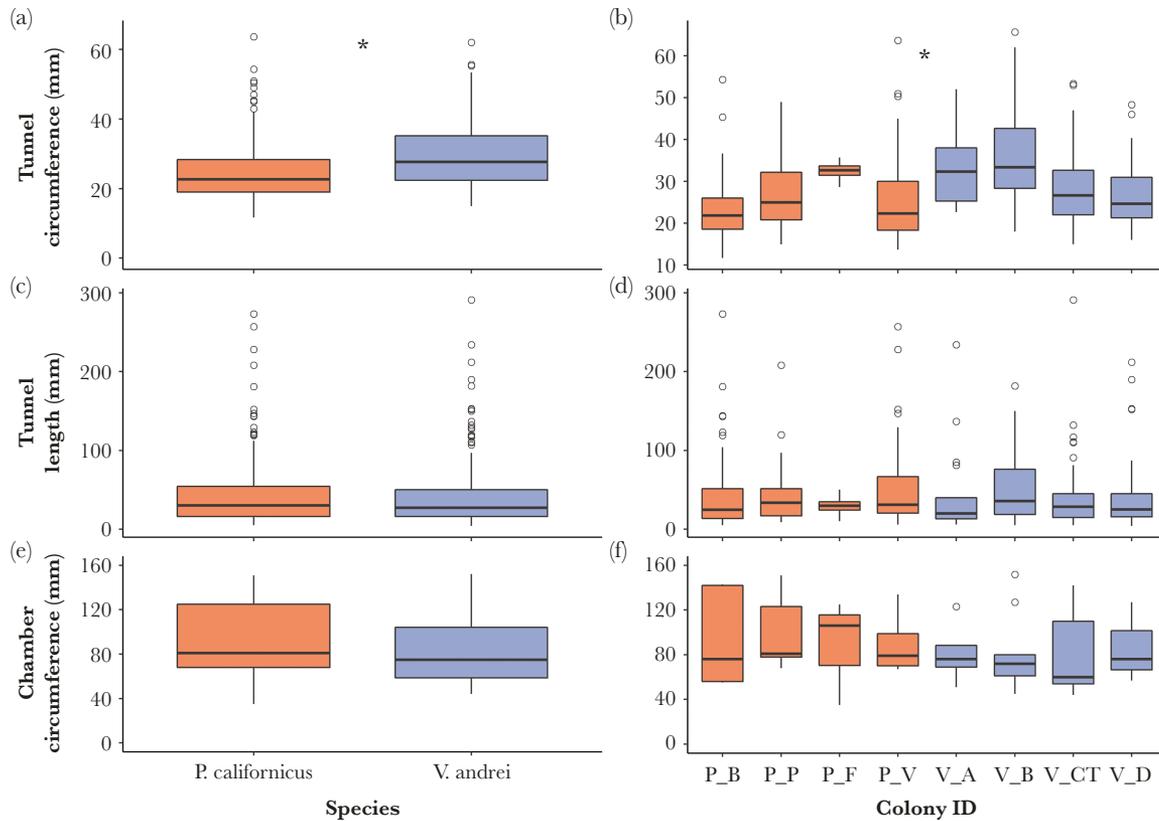


Figure 2

Effects of species and colony on tunnel and chamber measures. Differences between the two species, *P. californicus* (orange) and *V. andrei* (blue) (A, C, E), and the different source colonies within each species (B, D, F) in the circumference of tunnel segments (A, B), the length of tunnel segments (C, D), and the circumference of chambers (E, F). Asterisk denotes statistically significant differences ($P < 0.05$) based on an ANOVA (Table S3). Here, and in all following plots, boxes indicate interquartile ranges, bold lines inside the boxes are the median, whiskers extend to 1.5 times the interquartile range, and open dots denote outliers.

Table 1

Full statistical output of ANOVA

Response variable	Explanatory variable	Sum Sq	DF	F-value	P-value
Tunnel segment circumference	Species	2.805	1	31.272	<0.0001***
	Colony ID (nested in species)	3.177	6	5.903	<0.0001***
	Temperature	0.0234	1	0.261	0.61
	Humidity	0.08	1	0.889	0.346
Tunnel segment length	Species	0.439	1	0.628	0.429
	Colony ID (nested in species)	6.393	6	1.523	0.169
	Temperature	0.064	1	0.091	0.763
	Humidity	0.250	1	0.357	0.551
Chamber circumference	Species	3390	1	2.224	0.146
	Colony ID (nested in species)	1860	6	0.177	0.981
	Temperature	14	1	0.008	0.930
	Humidity	66	1	0.038	0.847
Nest size	Species	0.050	1	0.015	0.904
	Colony ID (nested in species)	58.7	6	2.923	0.031*
	Temperature	0.147	1	0.044	0.836
	Humidity	1.17	1	0.349	0.561

Values in bold indicate statistical significance: one asterisk denotes a P -value below 0.05, three asterisks denotes P -values below 0.001.

circumference: $F = 1.558$, $df = 1$, $P = 0.213$, Figure 4B, Table S3; tunnel length: $F = 0.357$, $df = 1$, $P = 0.551$, Figure 4D, Table 1; chamber circumference: $F = 0.038$, $df = 1$, $P = 0.847$, Figure 4F, Table S5). Interestingly, both species dug significantly more nests with

cycles in hot compared with cold temperatures (Logistic regression: temperature: $Chisq = 4.99$, $df = 1$, $P = 0.026$, Figure 5A; Table 2). However, we did not detect a significant effect of humidity on the number of nests with cycles (Logistic regression: humidity: $Chisq = 0$,

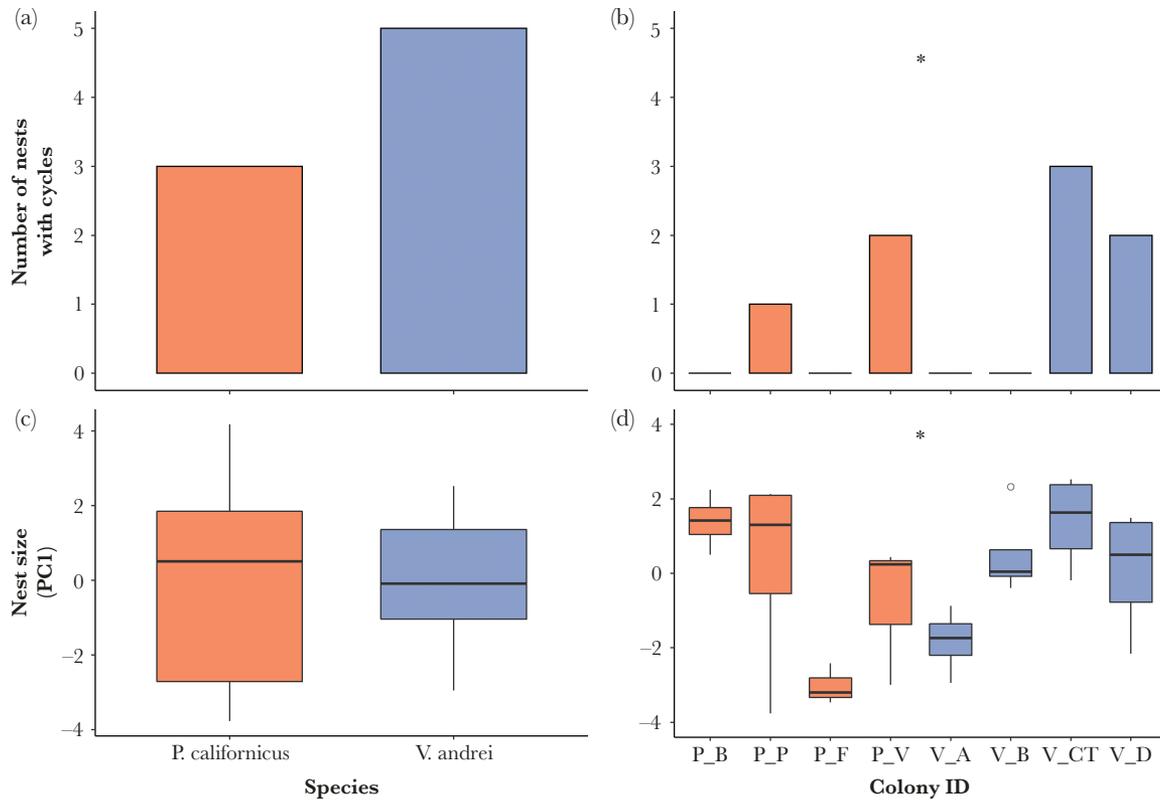


Figure 3

Effects of species and colony on global nest measures. Differences between the two species *P. californicus* (orange) and *V. andrei* (blue) (A, C) and the different source colonies within each species (B, D) in number of nests with cycles (A, B) and nest size (PC1) (C, D). Asterisk denotes statistically significant differences ($P < 0.05$) based on a logistic regression (C) or ANOVA (D).

Table 2

Full statistical output for logistic regression on number of nests with cycles

Variable	Chisq	DF	Pr (>Chisq)
Species	0.474	1	0.491
Colony ID (nested in species)	16.644	6	0.011*
Temperature	4.988	1	0.026*
Humidity	0.000	1	1.000

Values in bold indicate statistical significance: one asterisk denotes P -values below 0.05.

$df = 1$, $P = 1.0$, Figure 5B, Table 2). Finally, we did not detect a significant effect of temperature or humidity on nest size (ANOVA: temperature: $F = 0.046$, $df = 1$, $P = 0.833$, Figure 5C; humidity $F = 0.485$, $df = 1$, $P = 0.494$, Figure 5D; Table 1).

DISCUSSION

Our study shows that the intrinsic factors we examined, colony and species, have a stronger effect on nest structure than the extrinsic factors that we examined, temperature and humidity. Intrinsic factors contributed to differences in tunnel and global nest measures: colonies and species differed in tunnel circumference (Figure 2A,B), and colonies differed in nest size and the number of nests with cycles (Figure 3B,D). The extrinsic factors we examined had only a small impact on nest-level measures, with more nests with cycles dug in hot compared with cold temperatures (Figure 5A).

We found a strong influence of the intrinsic factors we measured, colony and species, on harvester ant nest structure, impacting multiple structural elements. *Veromessor andrei* workers dug wider tunnels than *P. californicus* workers (Figure 2A), which can be explained by the longer bodies of *V. andrei* workers (Fig. S1). Longer bodies might require wider tunnels to allow effective turning. Interestingly, head width does not differ between the two species (Fig. S1) suggesting that head width and mandible gape do not have a large impact on tunnel width. The differences we observed among colonies in tunnel circumference, nest size, and number of nests with cycles (Figures 2B, 3B,D) likely reflect colony-specific characteristics that may be influenced by genetic differences, age, or maternal effects. Indeed, colony age influences nest size in other ant species (Tschinkel 2004; Wagner et al. 2004) thus it is possible that differences we observed between colonies in nest size (Figure 3D) reflect differences in colony age or size. However, all nests in this study were dug by the same number of ants and we had no information on colony age in our study populations. The nests of the colonies in our study were at least 50 m from one another and so come from different queens, representing different genetic backgrounds (De Vita 1979; Brown 1999). While we controlled for the amount of time ants were allowed to dig in our experiments, it is possible that colonies differ in their developmental history or the microhabitat in which they reside, potentially influencing the propensity of the workers to dig in the lab. Resource availability, population density, and colony maturity can all influence the collective behavior of colonies (Bengston and Jandt 2014). Furthermore, colonies may differ in the average size of workers (Kwapich et al. 2018), thus it

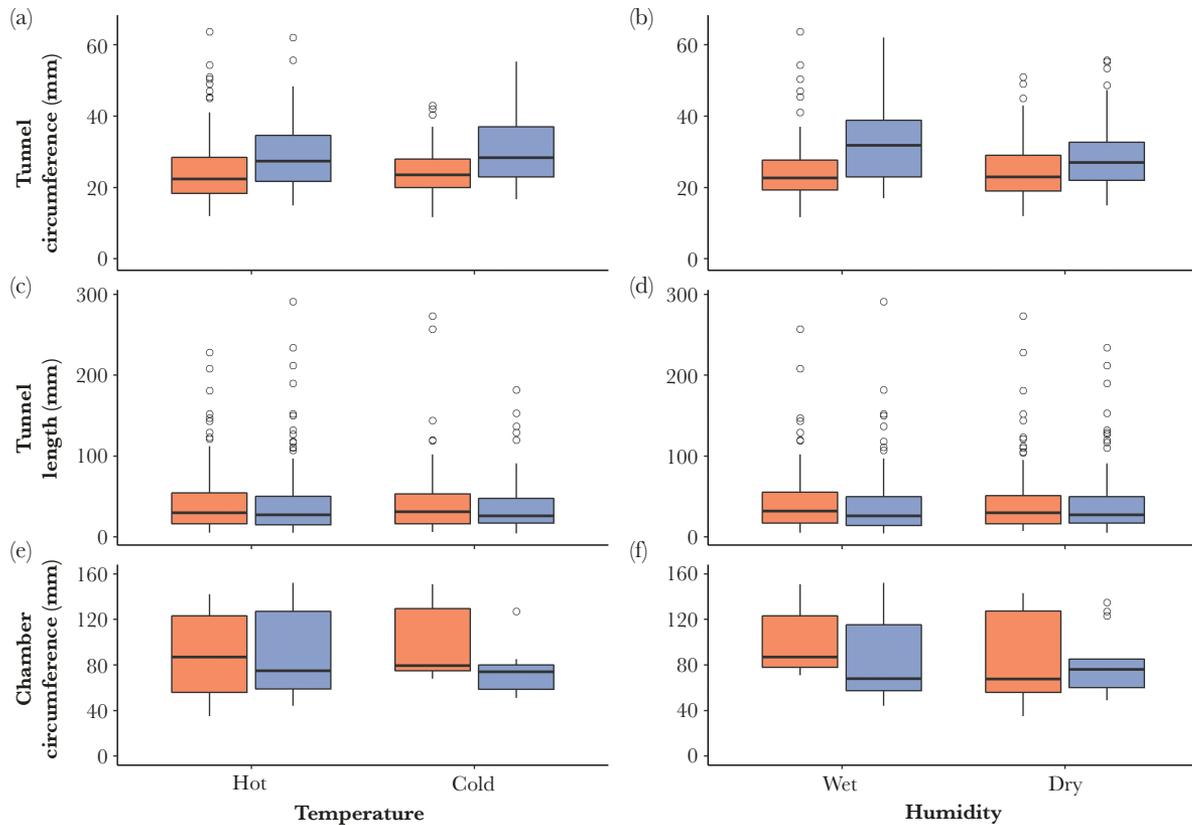


Figure 4

Effect of temperature and humidity on tunnel and chamber measures. Differences between nests dug in two different temperatures (A, C, E) and two different humidities (B, D, F) in tunnel segment circumference (A, B) tunnel segment length (C, D) and chamber circumference (E, F). Nests dug by *P. californicus* are in orange (left box in each pair) and by *V. andrei* are in blue (right box in each pair).

is possible that differences among colonies in tunnel circumference are due to different worker sizes. Overall, the differences in nest structure that we observed among colonies suggest that there are intrinsic, colony-specific characteristics that influence an extended phenotype. Further work is needed to identify the specific intrinsic factors that produce structural differences among colonies. In particular, it is important to determine whether intrinsic factors are inherited or heritable to uncover the potential evolutionary pressures on extended phenotypes.

The differences we observed among colonies and species in the nests they dig may have fitness consequences through the effects of nest structure on colony behavior. For example, nests with more cycles facilitate rapid recruitment to food (Pinter-Wollman 2015). Thus, differences among colonies in propensity to dig cycles (Figure 3B) might lead to differences in foraging success. Tunnel width might facilitate effective recruitment by allowing more interactions that regulate foraging behavior (Pinter-Wollman et al. 2013), thus differences in tunnel widths among colonies could further lead to differences in foraging. Furthermore, the mass recruitment to foraging (Johnson 2000) and frequent nest relocation activity (Brown 1999; Pinter-Wollman and Brown 2015) of *V. andrei* may require wide tunnels to facilitate rapid flow of ants, material, and interactions to exchange information. In contrast, the solitary foraging (Johnson 2000) and infrequent nest relocation (De Vita 1979) of *P. californicus* may not require large tunnel circumferences to facilitate mass movement. We did not detect differences among colonies or species in tunnel length and chamber size, which suggests that these

structural elements might be conserved across species, perhaps because of a functional importance they have. Indeed, a cross-species comparison shows that chamber size is highly conserved across species, regardless of colony size, or phylogenetic origin, similar to the conserved size of cells in bodies of multicellular organisms (Miller et al. 2022).

We found limited influence of the extrinsic factors that we tested, temperature and humidity, on harvester ant nest structure. Only the number of nests with cycles significantly increased in hot temperature (Figure 5A). Cycles facilitate rapid recruitment to food (Pinter-Wollman 2015); thus, colonies may dig nest structures that lead to faster recruitment in high temperatures if food is more ephemeral in warm conditions. Indeed, in warmer weather harvester ants scavenge on dead insects in addition to collecting seeds (Belchior et al. 2012). Such dead insects may be taken by other animals thus it is possible that more rapid recruitment in warmer weather allows harvester ants to capitalize on these ephemeral resources. Furthermore, cycles may facilitate air flow throughout the nest, serving a thermoregulatory function (Korb 2003) and warmer conditions may require more airflow to cool down the nest. Closing and opening cycles potentially require minimal work in the form of opening or closing tunnels, therefore such renovations could be an efficient way for colonies to adjust their nest to changing environmental conditions. It would be interesting to examine if over the course of the year, harvester ants renovate their nests to modify the number of cycles to either expedite recruitment or regulate internal temperature as the outside temperatures

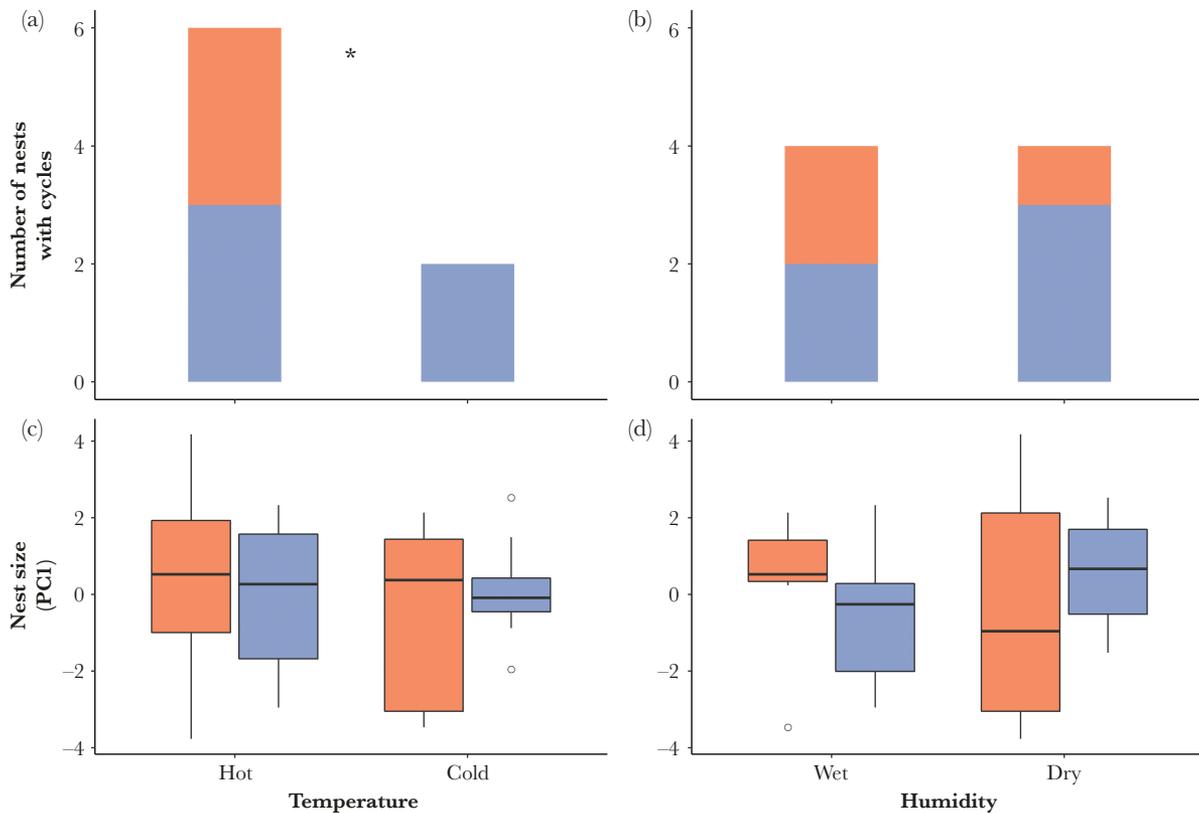


Figure 5

Effects of temperature and humidity on global nest measures. Differences between nests dug in two different temperatures (A, C) and two different humidities (B, D) on the number of nests with cycles (A, B) and on nest size (C, D). Nests dug by *P. californicus* are in orange (left box in each pair) and by *V. andrei* are in blue (right box in each pair). Asterisk denotes statistically significant differences ($P < 0.05$) based on a logistic regression.

change. We did not detect an effect of temperature or humidity on other nest features. However, differences in the nest size of the two species seemed to have an opposite relationship with humidity. *Pogonomyrmex californicus* nests tended to be larger under wet conditions, whereas *V. andrei* nests tended to be larger under dry conditions. The foraging activity of both *V. andrei* (Pinter-Wollman et al. 2012) and other *Pogonomyrmex* species (Gordon et al. 2013) increases with humidity. Thus, it is possible that changes to nest structure in response to humidity help facilitate changes in foraging behavior. Further work with a larger sample size could shed light on whether the nest structure of different harvester ant species indeed responds in different ways to humidity. We did not find a significant effect of temperature or humidity on any other nest structure, suggesting that the other features we measured are not sensitive to temperature and humidity. These findings also suggest that while high temperatures may lead to increased worker activity (Drees et al. 2007; Tizón et al. 2014), an exposure to high temperatures for an entire week might not impact worker activity in a way that influences their digging behavior. There may be species-specific effects of temperature on nest structure because temperature has an impact on the structure of *Formica podzolica* ant nests (Sankovitz and Purcell 2021). Perhaps measuring other nest features, examining other extrinsic effects, sampling colonies from different populations and microhabitats, or allowing the ants to dig for a different duration would reveal differences in nest structure that are mediated by the environment.

Our results suggest that extended phenotypes are shaped more strongly by the intrinsic factors we tested rather than the

extrinsic factors we examined. Like many physical and physiological phenotypes, intrinsic differences may contribute more strongly to differences among individuals and species than environmental conditions. Changes caused by extrinsic factors suggest plastic traits that can develop over time and adjust to rapidly changing conditions. However, for extended phenotypes to evolve by natural selection, differences among individuals in their extended phenotype must be both heritable and linked to fitness, rather than inherited. Our findings that intrinsic factors, like genotype and evolutionary history, have a greater impact on extended phenotypes than extrinsic factors, like temperature and humidity, suggest that there is a potential for natural selection to act on extended phenotypes, as it does on other morphological traits. Further work investigating other intrinsic and extrinsic variables in this and other systems will expand our understanding of which extrinsic conditions and which intrinsic features have the largest impact on extended phenotypes.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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AUTHORS' CONTRIBUTIONS

S.O, E.H.L, and N.P.W designed the study; S.O and E.H.L carried out the experiment; S.O, E.H.L, and N.P.W analyzed the results; D.D., S.O and E.H.L digitized the nest casts; S.O and E.H.L wrote the first draft of the manuscript; S.O, E.H.L, D.D. and N.P.W prepared the manuscript.

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Ethics: The research was conducted in compliance with guidelines for the care and study of invertebrates.

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