

Environmental influences on bioturbation and nest architecture of montane ants

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Abstract:

Social insects are among the most abundant arthropods encountered in terrestrial ecosystems and serve many functional roles that are beneficial to humans. One prominent ecosystem service that subterranean ants provide is the maintenance of healthy soil through aeration, decomposition, and nutrient cycling. The effect of ant colonies on soil properties has been studied for decades, yet little is known about ant nest properties like architecture due to the difficulty of observing these belowground patterns. Furthermore, many ant species span environmental gradients, and their behavior is largely dictated by the climatic and landscape features of their specific habitats. In this study, I explored how abiotic factors shape what can be considered as an ‘extended phenotype’ of ant colonies - nest architecture. Using a controlled laboratory setting, I quantified the nest structure of montane *Formica podzolica* ant colonies spanning a significant elevation range (~3000 ft) in Boulder County Parks & Open Space (BCPOS). Colonies exposed to a high-temperature treatment built deeper nests than those exposed to a low-temperature treatment. Colonies responded in the same ways regardless of their elevational origin. These results indicate that ant nests may be a plastic extended phenotypic trait that can change depending on the climatic environment of the nest, which may help colonies survive in adverse conditions. Further, this study reveals that *F. podzolica*, a common alpine ant throughout BCPOS, will likely be able to adjust to drastic changes in air temperature through the depth of its nests and, therefore, continue to maintain soil ecosystems in the face of climate change.

Key words: ant, nest, *Formica podzolica*, Boulder County, soil, temperature

Introduction:

Numerous studies have measured the above-ground impacts of ants (Debruyn & Conacher 1990; Sankovitz et al. 2019), but subterranean nest architecture and bioturbation (the mixing of soil by ants) have received comparatively little research attention, likely due to the difficulty of observing and measuring these below-ground aspects of ant life. However, the nests of ants have undoubtedly contributed to their ecological success and can be considered as an extended phenotype, providing benefits to survival in terms of shelter and defense (Minter et al. 2011; Dawkins 2016). Further, they are significant aspects of soil modification that can affect the surrounding vegetation (Frouz & Jilková 2008). The building of tunnels and chambers increases soil porosity (McCahon & Lockwood 1990) and balances water infiltration with environmental moisture (Cammeraat et al. 2002). Additionally, continuous heaping and mixing of soil may support the persistence of some plants that may otherwise suffer from competition (Dostal 2007). During excavation, ants move significant amounts of soil both upward and downward (contrary to popular belief), mixing layers of nutrients (Halfen & Hasiotis 2010). Although most of the ecosystem impacts of ants we perceive are at the soil surface level, much of the soil transformation occurs below ground.

Ants mix and displace soil in a manner that supports the existence of other organisms, but their primary goal is creating a space for their colony to live within. A complete understanding of why ants are soil engineers requires us to look beyond just their impacts on other organisms, and also explore how the modifications they produce can help their own colonies adapt to their particular environments. Since ants alter their environment and control nutrient and substrate movement, they are likely to modify natural selection pressures that are present in their local selective environment (Odling-Smee et al. 2003). It is therefore important to establish the link between the ecological requirements of ecosystem engineers and their impacts on ecosystem functioning. Jones et al. (1994,

1997) defined the ‘extended phenotype engineers’, building on the ideas of Dawkins (1982), as organisms creating structures or effects that directly influence the fitness of colonies (in the case of social insects). According to this definition, ants can be considered as extended phenotype engineers because their mounds have direct and positive feedback effects on the colonies through the maintenance of microclimate and protection of the population from enemies (Turner 2009).

The architecture of nest structures allows ants to regulate their environment to some extent. Thatch-mound-building ants modify the properties of their nest structures in order to regulate the microclimate (Rosengren et al. 1987). Yellow meadow ants (*Lasius flavus*) respond to taller vegetation by building more massive mounds with soil excavated from deeper soil layers, thereby changing the shape of the mound to optimize the collection of solar radiation (Blomqvist et al. 2000). The large colonies of red *Formica* wood ants build elaborate mounds constructed of plant materials and mineral soil, reducing the loss of heat and moisture (Hölldobler & Wilson 1990). Harvester ants (*Pogonomyrmex*) create and maintain vegetation-free zones around their nests by removing debris and clipping the vegetation, reducing transit time for foragers, decreasing the risk of fire and predation, and increasing exposure to solar radiation (MacMahon et al. 2000). These examples show that nests can lead to the decrease of environmental hazards and the optimization of colony development. As Jones et al. (1994) postulated, the nest structures of ants cannot be considered as accidents, but rather as responses to their environmental surroundings and thus an extended plastic phenotypic trait that allow ants to occupy many different habitats. As the needs of colonies vary with time, the properties of nests likely also change (Jouquet et al. 2006). Therefore, the influence of ants on soil physical and chemical properties may partly be explained by their habitat.

The literature on subterranean nest architecture and bioturbation is dominated by studies conducted in only one habitat, yet many ant species span a variety of habitats, some of which are

rapidly changing with climate and anthropogenic disturbances. With their widespread geographic success, ants may be altering nest architecture and bioturbation patterns to better survive in a wide range of environments, such as those exhibited across BCPOS. Much work has demonstrated ant behavioral and morphological phenotypic variation along environmental gradients (Cushman et al. 1993; Michaelangeli 2003; Traniello et al. 1984), and in this study I build on this work to investigate the extent to which the extended phenotype of the nest is plastic and varies with environment. To investigate how key environmental factors influence the excavation patterns and nest architecture of native montane ants, I collected worker *Formica podzolica* ants from both low- and high-elevation colonies in BCPOS and housed them in transparent nest boxes in a laboratory setting. I allowed the ants to excavate nests under treatments of soil type and temperature. This allowed me to assess whether they build nests differently under various abiotic conditions, which gives us a clue as to how they may be able to survive under changing climates and illuminates the level of conservation attention needed for these insects in BCPOS.

Methods:

Study Sites

I utilized montane *F. podzolica* ant colonies spanning an elevation of ~7000-10000 ft in Boulder County. Low elevation sites included Platt-Rogers Memorial Park (39.98° N, 105.44° W, ~7100 ft), Mud Lake Open Space (39.98° N, 105.51° W, ~8,250 ft) and Reynolds Ranch (40.17° N, 122.24° W, ~7200 m), and my high elevation sites included areas surrounding the Sourdough Trail (a hiking trail through a closed-canopy pine forest with limited undergrowth diversity; 40°02' N 105°31' W, ~9800 ft) and Brainard Lake Recreation Area (40.08° N, 105.57° W, ~10500 ft) (Figure 1).

Study organism

Formica podzolica nests occur in pine and aspen stands in Colorado and throughout the Rocky Mountain region, at altitudes up to 3000 m. This species constructs conspicuous soil mounds, which can exceed 2 m in diameter and estimates of colony size range from 5000 to 100,000 workers (Deslippe & Savolainen 1995). Workers tend aphids for honeydew, scavenge, and prey on a wide variety of invertebrates from March to October (Deslippe & Savolainen 1995). Winged, sexual individuals mate outside their nests from July to September, after which males die and females start new colonies (Deslippe & Savolainen 1995). About one-third of *F. podzolica* colonies contain a single queen, while most have more than one queen, with low average nestmate relatedness (DeHeer & Herbers 2004).

Experimental Design

During June-September 2019, I collected 100 *F. podzolica* workers using forceps from ten *F. podzolica* colonies from both low and high elevations, housed them in 20 transparent nest boxes (18"x20"x1.5") in a laboratory setting, and allowed them one week to excavate nests (Figure 2). I provided them with water and fed them a diet of sugar water. I tested treatments of temperature and soil type. In the soil type experiment, I introduced colonies to either sandy or loamy soil at a temperature of 78°F. I only carried out one trial of 20 colonies for this experiment, because the ants were unable to build nests in the sandy soil. In the next experiment, I tested the effects of temperature on bioturbation and nest architecture, applying a high temperature treatment to 10 boxes (88°F, the July high temperature averaged from 1981-2010 in Nederland at ~8000 ft; NOAA 2019) and a cooler temperature treatment to the other 10 boxes (67°F, the July high temperature averaged from 1981-2010 in Ward at ~9,400 ft; NOAA 2019). The room that contained the boxes was kept at 60°F. I

monitored temperature with three iButtons per box: one on the soil surface, one in the soil at half the depth of the box, and one in the soil at the bottom of the box. I replicated the temperature experiment three times for a total of 52 colonies (some of the colonies had complications, such as high death rates or failure to excavate).

I kept the nest boxes in the insulated basement of a residential house in Boulder, which mimics subterranean soil temperatures. The glass walls of the boxes were kept dark with black fabric to mimic a subterranean habitat. Four temperature chambers encapsulated five boxes each from the soil surface up, with the majority of the area of the nest boxes extending below the chambers and in contact with the ambient air temperature (Figures 3 and 4). Two chambers had the high-temperature treatment, and the other two chambers had the low-temperature treatment. The heat was applied via a thermal mass consisting of a concrete mortar mix surrounding radiant heating tubing, which was connected to a temperature-controlling outlet. I monitored soil temperature using iButton temperature sensors at various depths.

To examine bioturbation, I added a thin layer of colored sand every 10 cm (a different color for every layer) to one trial of 20 colonies (similar to Halfen & Hasiotis 2010). The aim of these colors was to allow me to assess the movement of the soil layers visually, but the ants uniformly stopped digging once they reached the first layer of sand (Figure 5). This was unexpected, as the harvester ants used in Halfen & Hasiotis 2010 were not sensitive to layers of various substrates. I excluded these sand layers from the rest of the experimental trials of nest architecture so as not to influence the ways the ants built their tunnels.

In an attempt to create layers of colored substrate that were the same density as the rest of the soil (so as not to influence the tunnel structure), I added food dye to soil of the same loamy type and baked it until dry, which dyed the soil various muted colors. I layered this in a couple nest boxes

to test the bioturbation experiment again, and although the ants did not appear to alter their digging behavior when they reached a colored layer, the moved soil particles were indistinguishable from the surrounding soil (the colors did not contrast enough with the brown soil).

Nest Architecture Measurements

After the excavation period of each trial, I took photographs of each side of every box to measure attributes of nest architecture. I quantified nest architecture by measuring the total number and depth of chambers at experiment completion.

Data Analysis

I performed all statistical analyses in R (V 5.3.5, The R Foundation for Statistical Computing). I used the *aov* and *TukeyHSD* functions from the stats package (Miller 1981; Chambers et al. 1992) to fit an analysis of variance (ANOVA) model and compute Tukey honest significant differences (Tukey HSD) between the high and low colonies tested in high and low temperatures. In these models, number of tunnel branches and nest depth (in) were the dependent variables and the combination of colony origin elevation and temperature treatment was the independent variable.

Results:

Overall, colonies exposed to the high-temperature treatment built deeper nests than those exposed to the low-temperature treatment (Figure 6). The Tukey HSD test results for comparisons between the high elevation colonies exposed to the high-temperature treatment (HH), high elevation colonies exposed to the temperature treatment (HL), low elevation colonies exposed to the high-temperature treatment (LH), and low elevation colonies exposed to the low-temperature

treatment (LL) are as follows: HL-HH: $p=0.0862$; LH-HH: $p=0.9899$; LL-HH: $p=0.0156$; LH-HL: $p=0.0541$; LL-HL: $p=0.9669$; LL-LH: $p=0.0094$. The ANOVA indicated significant differences between the groups ($F=5.713$, $p=0.002$).

There was no significant difference in the number of tunnel branches across colony origin elevation and temperature treatment (ANOVA: $F=1.207$, $p=0.317$) (Figure 7). The Tukey HSD test results for comparisons between the high elevation colonies exposed to the high-temperature treatment (HH), high elevation colonies exposed to the temperature treatment (HL), low elevation colonies exposed to the high-temperature treatment (LH), and low elevation colonies exposed to the low-temperature treatment (LL) are as follows: HL-HH: $p=0.995$; LH-HH: $p=0.9999$; LL-HH: $p=0.4618$; LH-HL: $p=0.9958$; LL-HL: $p=0.3757$; LL-LH: $p=0.4939$.

Discussion:

Regardless of the original elevation of the colony, those exposed to the high-temperature treatment built deeper nests than those exposed to the low-temperature treatment. This difference in depth could be due to a greater buffering distance needed when the surface air temperature is hot, whereas a cooler, milder temperature may allow the ants to remain near the surface of the soil within more superficial nests. The high-elevation colonies had the same response as the low-elevation colonies: they built nests of markedly different depths depending on the temperature. This result indicates the ability of *F. podzolica* ants to adjust to their abiotic environment and suggests that they are able to adjust in this way across their range. It also suggests that nest architecture is somewhat of a plastic trait. The extended phenotype of the nest may allow for better colony survival if the ants are able to quickly adjust to changing air temperature. Additionally, the ability of colonies to build different nests depending on their environment may be a clue into the

widespread geographic success of ants and suggests that they will fare better than other organisms under changing climate pressures.

The absence of significant differences in the number of tunnel branches opens up many more questions about nest structure. Although the number of tunnel branches played no apparent role in helping colonies adjust to their abiotic environment, this metric may be a result of individual worker decision-making, collective decision-making, or stochasticity.

The ants' behavior when I tried layers of colored sand may be explained by the different soil densities or texture. The ants dug down to the first layer and then dug along the layer (and no deeper). This suggests that substrate layers play an important role in nest architecture, in addition to air temperature. It is likely any soil layer that has a different density than the topsoil layer will likely influence a change in nest architecture. Further, the observation that none of the ants could dig in sandy soil suggests that soil type is an important abiotic factor that constrains the range of *F. podzolica*; they may need loamy soil for nesting because sandy soil may be too dry and easily collapsible.

This study is limited in its design and there is much room for future research on the topic of how ants impact soil. (1) My nest box design only allowed for the observation of two-dimensional nests, so the utilization of space in which three-dimensional nests could be observed would be more ideal. (2) My sampling was limited to one generalist species, so nest architecture should be examined in many more ant species that span elevational gradients. Future studies should collect other ant species with different adaptive traits, especially those which are habitat specialized, in order to assess the effect of elevation and temperature on non-generalist species and implications of abiotic factors on ant species resiliency and adaptive capacity. Finally, (3) the ways in which bioturbation and soil type play a role in ant nest architecture, and how they vary across the range of

an ant species, are topics I attempted to address in this study but failed at - a more effective experimental design to address these questions is much needed.

Overall, this study elucidates how air temperature, a key environmental factor, influences subterranean behavior that is integral to the functional role of ants as soil ecosystem engineers. Additionally, it suggests that *F. podzolica* ants need loamy soil for nest construction, and any soil layer that has a different density than the topsoil layer will likely influence a change in nest architecture. Ants live throughout Boulder County Parks & Open Space and are modifying soil virtually everywhere throughout the land. This study reveals that *F. podzolica*, a common alpine ant throughout Boulder County, may be able to adjust to drastic changes in air temperature through the depth of its nests and, therefore, continue to maintain soil ecosystems in the face of climate change. Although temperature is a significant factor in an ant's habitat, there are other abiotic factors that likely influence ant nest architecture, such as seasonal precipitation. Future studies should investigate the role of precipitation in the patterns and plasticity of nest architecture and bioturbation. This study also suggests that subterranean ant nest architecture is a plastic extended phenotypic trait that might contribute to the widespread geographic success of this insect family, in Boulder County and beyond.

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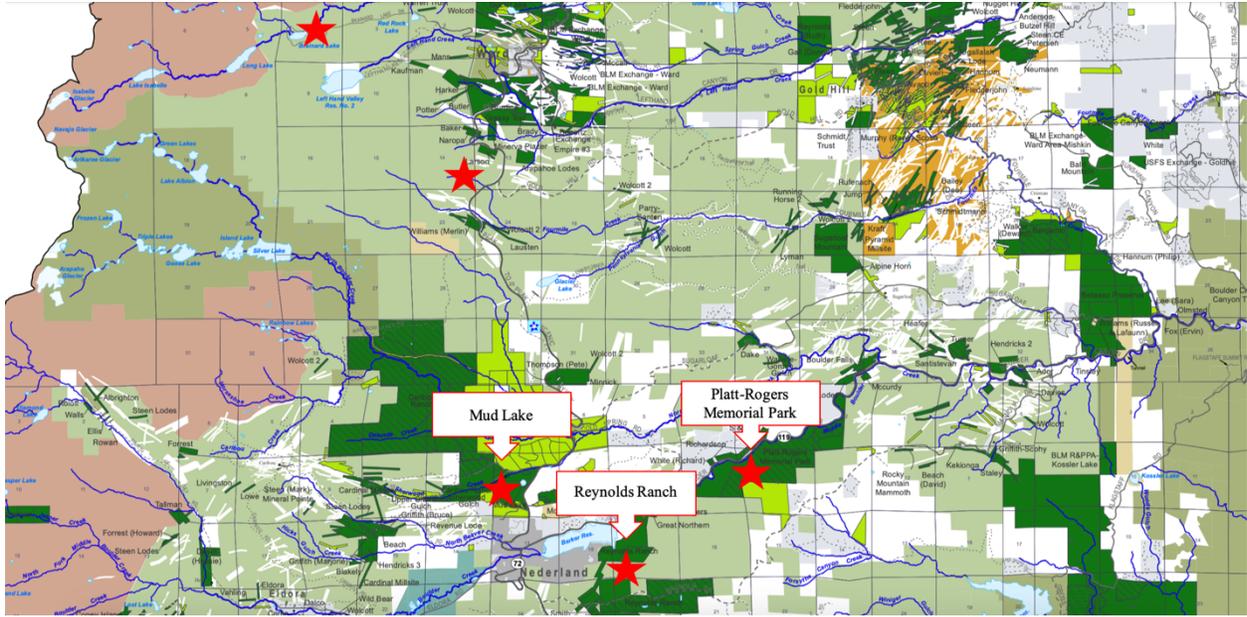


Figure 1: Map of study sites. Stars are located at study sites, and BCPOS properties are labeled.



Figure 2: Example of a nest built within a nest box.

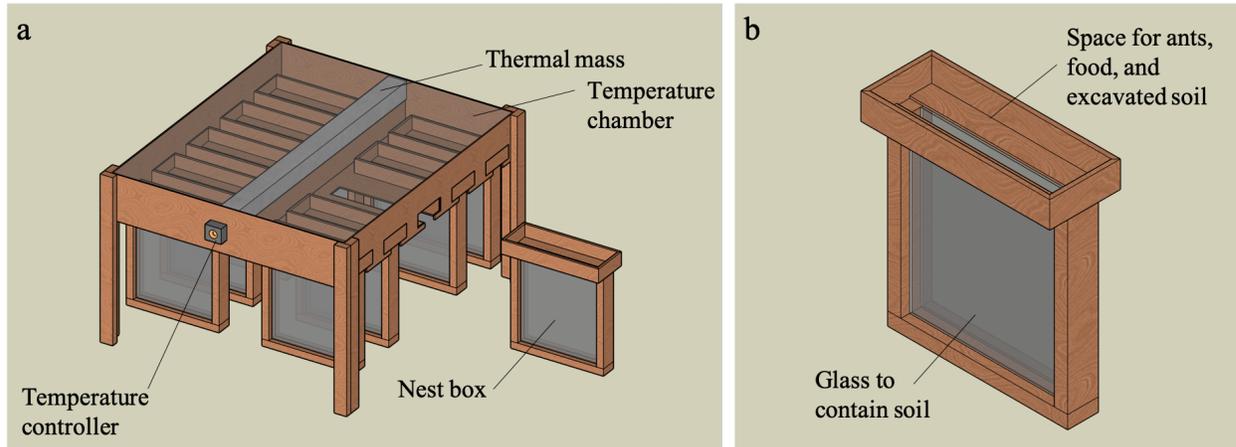


Figure 3: Layout of (a) two warming chambers containing five nest boxes each, with a concrete thermal mass separating them, and (b) one nest box. The temperature chambers were covered in the experiment with a plastic lid but are uncovered for the purposes of this diagram.

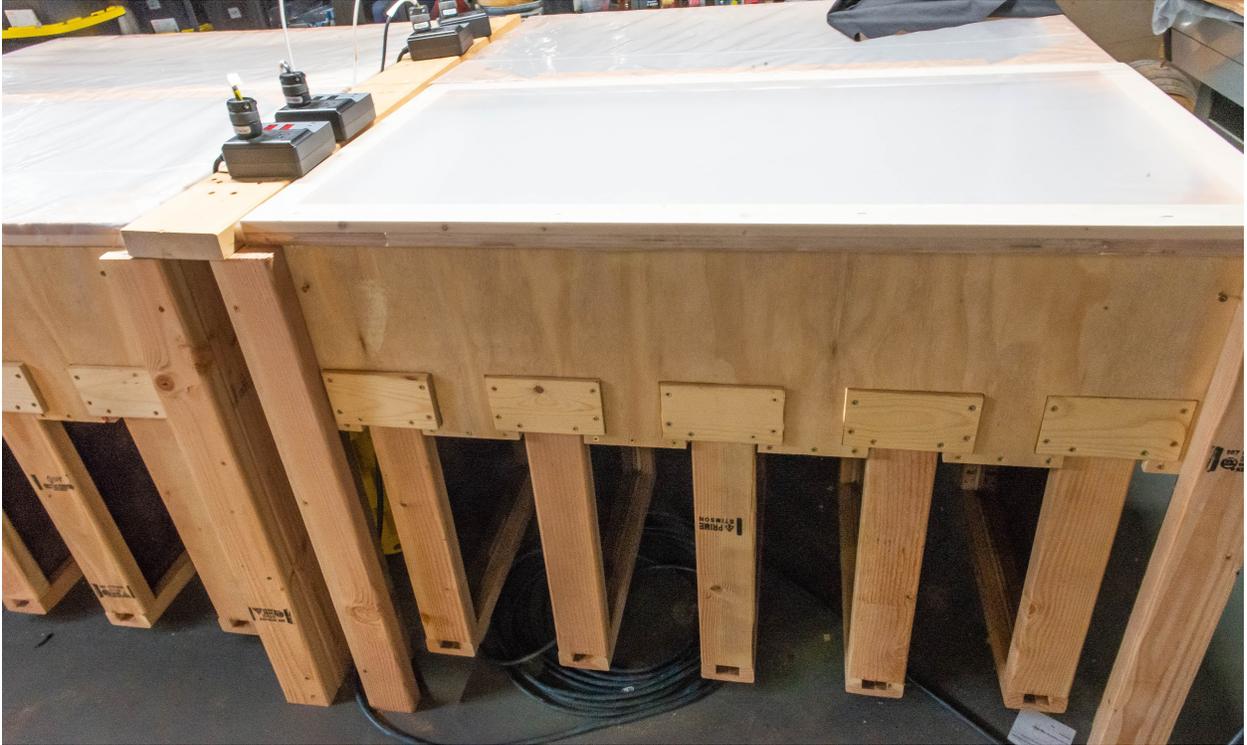


Figure 4: Photo of warming chambers with nest boxes hanging below. The black fabric used to keep the nests dark has been removed for the purposes of this photograph.



Figure 5: Example of tunnels built down to, and along, a layer of colored sand. The tunnels do not extend deeper into the box past this layer.

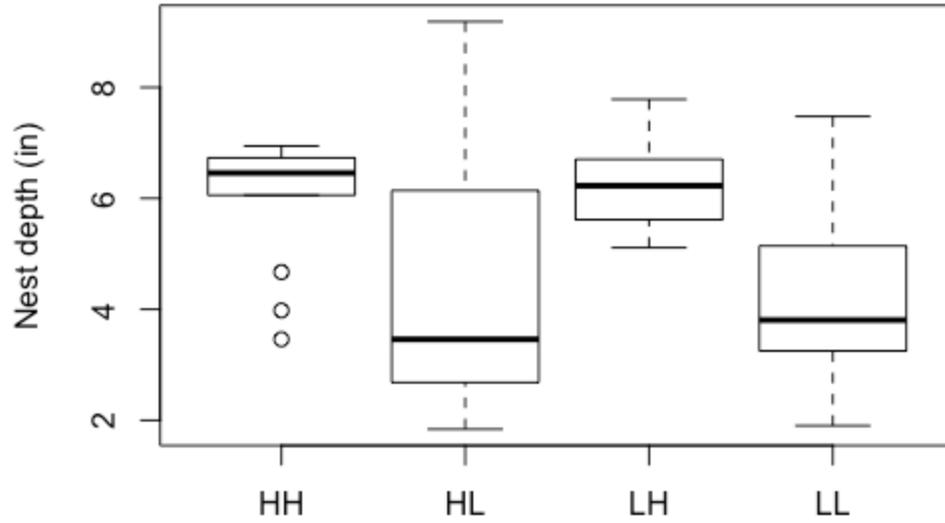


Figure 6: Nest depth (in) for high elevation colonies exposed to the high-temperature treatment (HH), high elevation colonies exposed to the low-temperature treatment (HL), low elevation colonies exposed to the high-temperature treatment (LH), and low elevation colonies exposed to the low-temperature treatment (LL).

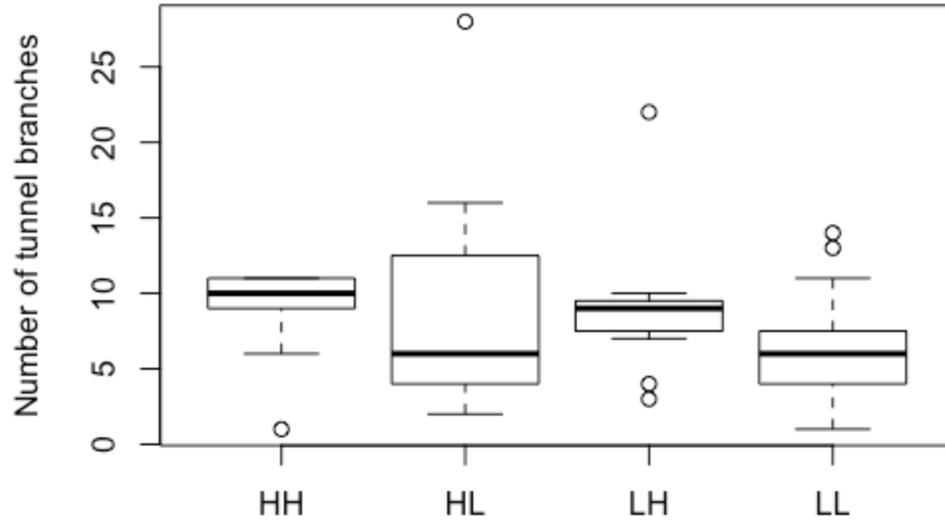


Figure 7: Number of tunnel branches for high elevation colonies exposed to the high-temperature treatment (HH), high elevation colonies exposed to the low-temperature treatment (HL), low elevation colonies exposed to the high-temperature treatment (LH), and low elevation colonies exposed to the low-temperature treatment (LL).