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UNIVERSITY OF CALIFORNIA
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An Interdisciplinary Approach to Understanding Environmental
Dynamics of Soil Ecosystem Engineering Ants

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Entomology

by

Madison Sankovitz

December 2022

Dissertation Committee:
      Dr. Jessica Purcell, Chairperson
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The Dissertation of Madison Sankovitz is approved:

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Committee Chairperson

University of California, Riverside

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Ana, Anica, Chris, Christine, Kaleigh, Millicent, Nate, Nikki, Sigrunn, and Susan for your true love and friendship. For me, the beauty of life is reflected in each of you. I love you all.

For Jay: On the days I shine brightest, your light is there in the soft hues of dusk and in the dawn of a new day. In my darkest moments, you are the full moon guiding the path to a better place. One of the greatest gifts of my life is knowing you.

Dedication

I dedicate this dissertation to young Madison, to whom I am always trying to find my way back. She knows what's up.

ABSTRACT OF THE DISSERTATION

An Interdisciplinary Approach to Understanding Environmental
Dynamics of Soil Ecosystem Engineering Ants

by

Madison Sankovitz

Doctor of Philosophy, Graduate Program in Entomology
University of California, Riverside, December 2022
Dr. Jessica Purcell, Chairperson

Organisms exhibit characteristics that can change depending on the environment and vary
across a species, especially those with expansive ranges. Due to eusociality, which has
led to proliferation throughout nearly every terrestrial ecosystem, many social insects
make ideal subjects for studying variation in how a species interacts with the
environment across its range. Soil-dwelling ants create and modify habitat as ecosystem
engineers while building nests that act as an extended phenotype, buffering the colony
against climate. Understanding how ants can persist in a wide range of environments and
developing a predictive understanding of their soil impacts across environmental
gradients will advance our knowledge of soil ecosystem function. Through soil sampling
in and around nests and a controlled laboratory experiment, I investigated the extent to
which climate influences nest architecture and the effects of nests on soil properties.
Additionally, I conducted genomic analyses to identify markers associated with climatic
heterogeneity in a widespread species, *Formica podzolica*. I found that nests
differentially affect soil chemistry across elevational gradients; at lower elevations, nest
soil had lower amounts of carbon and nitrogen than control soil, but at higher elevations,
the opposite pattern was present. Nest architecture is shaped by local adaptation and a

plastic response to temperature; while workers experiencing a high temperature excavated deeper nests than those experiencing a cooler temperature, I observed a significant interaction effect of natal elevation and temperature treatment on nest size and complexity. While these traits are plastic, genomic underpinnings may also influence ants' fitness and their impact on soil in different climates; genomic signatures of adaptation to temperature, precipitation, and seasonality were present across *F. podzolica*'s range, with one locus exhibiting a precipitation-associated alternative allele exclusively at the northern edge of the range. Combined, these studies suggest that *Formica* ants likely modulate soil properties differently across environmental gradients, their nests are shaped by a combination of plastic and locally adapted behaviors, and genomic variation may be a factor in adaptive potential, especially at range margins.

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INTRODUCTION

Background

Organisms are defined by specific characteristics: behaviors, physical traits, and genetic makeup. While commonly viewed as static, these characteristics are often highly dependent on the unique abiotic environment in which an individual lives. Variation in these characteristics can be an expression of phenotypic plasticity or genetic differences across individuals. As an example of phenotypic plasticity, genetically identical water flea clones can differ in their morphology depending on whether reared in the absence or presence of a potential predator (Stoks et al. 2016). Conversely, some populations of yellow warblers across North America are declining and have been identified as "genomically vulnerable" because of limited climate-associated genomic variation (Bay et al. 2018). Hence, a species' characteristics must be considered in the context of environmental variation across its range.

Range size influences the degree of genetic and phenotypic variation that might be expressed within a species and the patterns of a species' impact on its environment through ecosystem engineering and interspecific interactions. Understanding why some species have more extensive ranges than others and the causes and consequences of range limits are key topics in ecology and evolutionary biology (Gaston 2009). Organisms cannot survive in locations where environmental conditions are too harsh for their physical tolerances and capacities (Spicer and Gaston 1999). This assumption underlies any prediction of species' responses to environmental, especially climate, change. Populations near range edges are often assumed to have reduced genetic diversity and

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population sizes, which limit evolutionary potential. The extent to which this is a global pattern, and the context in which it occurs, remain poorly understood.

In addition to phenotype and genotype, organisms can be characterized by their conspecific and heterospecific biotic interactions. Similarly, these interactions likely vary across a species' range depending on the specific environment. One example of this environmental context-dependency concerns ecosystem engineers, organisms that alter their physical surroundings or resource dynamics (Jones et al. 1994). For example, beavers create entire wetlands out of streams. Because ecosystem engineers play an integral role in creating or altering their habitat, engineers' impact is likely to shift across elevational and latitudinal gradients. Those that most significantly impact their ecosystem in positive ways modify limiting resources or constraining microclimates (Crain and Bertness 2006). In physically severe ecosystems, small alterations could create hospitable habitats for organisms that would otherwise not be able to survive. For example, in semiarid habitats where dry soils restrict plant growth, nurse plants that cast a shadow over soils and confine water play a crucial role in the survival of neighboring plants (Aguiar and Sala 1994). Contrastingly, in physically mild environments where competitor and consumer pressure is often high, ecosystem engineers have been shown to alter the availability of limited resources and change competitive hierarchies. Examples include mussels that make nutrient-rich deposits in intertidal zones (Bertness 1984) and seagrasses that reduce water speed and facilitate sediment deposition (Thomas et al. 2000). A significant criticism of the ecosystem engineering concept is that all organisms alter their environments somewhat, so the distinction is unnecessary to our understanding

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of ecological communities (Reichman and Seabloom 2002a; 2002b; Wilby 2002).

However, ecosystem engineers specifically reduce restricting abiotic and biotic pressures

and expand distributional limits for many species; the goal is determining the context in

which this process plays a significant role in community growth (Crain and Bertness

2006).

Understanding how organisms interact with their environment in different habitats

across their range is an understudied but crucial area of evolutionary ecology.

Environmental context has important implications for understanding phenotype,

genotype, and biotic and abiotic interactions. In this dissertation, I contribute to our

understanding of this using ants (Formicidae) as a study organism. Due to their

eusociality, which has led to proliferation throughout nearly every terrestrial ecosystem

on Earth, many social insects make great subjects for studying variation in how a species

interacts with its environment across its range. Organisms that exhibit eusociality have

the following characteristics: cooperative brood care, overlapping generations, and divide

their labor into reproductive and nonreproductive groups (Hölldobler et al. 1990). The

advantage of eusociality is apparent in that social insects represent more than half of all

arthropod biomass (Hunt and Gadau 2016). Ants dominate soil ecosystems throughout

every landmass except Antarctica and some isolated islands. Ants make up an estimated

15-20% of the terrestrial animal biomass on Earth (Schultz 2000). This undeniable

ecological success can be largely attributed both to eusociality, which gives ants the

protection and prosperity of a colony-based lifestyle, and the subterranean-nesting

lifestyle of many species, which buffers the colony from weather, predators, and parasites

on the surface. Ants have evolved to live in some of the most extreme environments, from the arctic tundra to the Sahara Desert and every intermediate climate. They have an incredible diversity of species range sizes, from local to continental scales. Studying the variation in ant behavior, phenotype, genotype, and interspecies interactions can lend insight into why some ant species are constrained to one geographic area while others can persist across vastly different climates and landscapes.

Many ants are considered ecosystem engineers due to their outsized impacts on their surrounding habitats. Many species build intricate subterranean nests that have undoubtedly contributed to their success and are considered an extended phenotype (manifestations of genes that occur outside of the organism that possess those genes), providing survival benefits to colonies in terms of shelter and defense (Minter et al. 2012; Dawkins 2016). Ants alter the physical and chemical aspects of soil in and around their nests, significantly affecting plants, microorganisms, and other soil macroinvertebrates (Folgarait 1998). For example, ant nests have been found to harbor higher plant growth, bacterial numbers, and macroinvertebrate species density, abundance, and biomass than surrounding soil (Holec and Frouz 2006; Doblas-Miranda et al. 2009; Farji-Brener and Werenkraut 2015). They increase soil drainage and aeration through the formation of subterranean tunnels and incorporate nutrients into soil through food storage and the accumulation of feces and corpses (Brian and Brian 1978). Most studies on ecosystem engineering by soil-dwelling ants have found increased organic matter, phosphorus, potassium, and nitrogen in nests compared to nearby soil (Salem and Hole 1968; Czerwinski et al. 1969; Petal 1978; Mandel and Sorenson 1982). Additionally, the mixing

of soil (bioturbation) through nest excavation is often substantial. Out of all animals, only earthworms (~15000 g m-2 y-1) mix more soil than ants (~5000 g m-2 y-1), but ants are distributed more broadly (Paton et al. 1995), and ants are estimated to be as crucial in overall soil modification (Gotwald 1986).

A multitude of studies has made it clear that soil-dwelling ants create and alter habitat and modulate the availability of resources, impacting other organisms in significant ways. However, because ants dwell in soils ranging from tropical rainforest to arctic and alpine tundra, their impact as ecosystem engineers is likely to shift across environmental gradients such as latitude and elevation. In other words, their ability to regulate ecosystem function likely differs between extreme and clement environments. The capacity of these engineers in different environments has been addressed only on rare occasions (Wright and Jones 2004; Crain and Bertness 2005; Crain and Bertness 2006). Numerous ant species distributions span large latitudinal and elevational gradients, suggesting that they are well-adapted to a wide range of climates. Similarly vast in range size, *Formica podzolica* is a native ant that lives in montane habitats in the southern portion of its range (New Mexico and Utah) and in the boreal forest in the northern portion of its range (Alaska and Yukon Territory) (Janicki et al. 2016; Guenard et al. 2017). The impact of ants as ecosystem engineers is likely to shift across climatic gradients, and the soil services of a species may be vastly different across its range.

Understanding how ants can persist in a wide range of environments and, subsequently, developing an understanding of their soil impacts in different environments are essential steps in advancing our knowledge of soil ecosystem composition and

function, particularly in the context of changing climates. However, much is yet to be understood about ants' abilities as ecosystem engineers in different environments and how they can survive in dramatically different climates across, in some cases, vast species ranges. In this dissertation, I examine the environmental interactions, extended phenotype, and genomes of *Formica* ants within various environmental contexts. Specifically, I examine patterns of ant-soil interactions and the genomic underpinnings of their survival across variable habitats.

Study system

  Formica is a widespread and diverse ant genus, living in habitats from the warm temperate to subarctic zones of the northern hemisphere (Janicki et al. 2016; Guenard et al. 2017). As of 2021, *Formica* contains at least 172 species (Borowiec et al. 2021). These ants live in many habitats, including woodlands, shrublands, grasslands, cities, coasts, and swamps. In residential areas, they usually nest close to structures such as sidewalks, fences, or building foundations. *Formica* nests vary from uncomplicated cavities in soil to large mounds and can be found under stones or inside logs. Many species within the genus are known to be socially polymorphic; most species studied thus far are known to have some single-queen colonies (monogyne) and some multi-queen colonies (polygyne) (Fontcuberta et al. 2022; McGuire et al. 2022; Pierce et al. 2022; Purcell et al. 2021).

  For several reasons, *Formica* is an excellent genus for investigating ecological and genomic patterns of species across environmental gradients. First, the genus inhabits

a broad latitudinal and elevational range, from warm temperate to subarctic zones (it also extends into the tropics at high elevations south to Honduras). Many individual species also have ranges spanning diverse climates and landscapes. This geographical diversity allows for intraspecific comparisons of populations from vastly different habitats and the investigation of signatures of adaptation that may allow ants to prosper and be influential soil engineers across environmental gradients. Second, many species build subterranean nests, making them ideal candidates for the study of soil ecosystem engineering.

Research objectives

Broadly, the goals of this dissertation were to investigate the degree to which climate affects ant-soil interactions and to reveal how ants may be adapted to the various climates in which they live. For each chapter, I carried out either field sampling, behavioral experiments, or genomic analyses with North American *Formica* to:

I.   assess the extent to which environment influences the effect that *Formica* ants have on soil chemical and physical properties,

II. determine whether nest architecture is a plastic extended phenotype that changes with climate, and

III. reveal how a widespread ant species may be adapted to climatic heterogeneity using a landscape genomics approach.

References

Aguiar MR, Sala OE (1994) Competition, facilitation, seed distribution and the origin of patches in a Patagonian steppe. Oikos 70:26–34.

Bay RA, Harrigan RJ, Underwood VL, Gibbs HL, Smith TB, Ruegg K (2018) Genomic signals of selection predict climate-driven population declines in a migratory bird. Science 359:83–86.

Bertness MD (1984) Ribbed mussels and Spartina alterniflora production in a New England salt marsh. Ecology 65:1794–1807.

Borowiec ML, Cover SP, Rabeling C (2021) The evolution of social parasitism in *Formica* ants revealed by a global phylogeny. P Natl Acad Sci USA 118: e2026029118.

Brian MV, Brian MV (1978) Production Ecology of Ants and Termites. Cambridge University Press.

Crain CM, Bertness MD (2005) Community impacts of a tussock sedge: Is ecosystem engineering important in benign habitats? Ecology 86:2695–2704.

Crain CM, Bertness MD (2006) Ecosystem engineering across environmental gradients: implications for conservation and management. Bioscience 56:211–218.

Czerwinski Z, Jakubszyk H, Petal J (1969) The influence of ants of the genus *Myrmica* on the physico-chemical and microbiological properties of soil within the compass of anthills in the Strzeleckie meadows. Pol J Soil Sci 2:51–58.

Dawkins R (2016) The Extended Phenotype: The Long Reach of the Gene. Oxford University Press.

Doblas-Miranda E, Sánchez-Piñero F, González-Megías A (2009) Different microhabitats affect soil macroinvertebrate assemblages in a Mediterranean arid ecosystem. Appl Soil Ecol 41:329–335.

Farji-Brener AG, Werenkraut V (2015) A meta-analysis of leaf-cutting ant nest effects on soil fertility and plant performance. Ecol Entomol 40:150–158.

Folgarait PJ (1998) Ant biodiversity and its relationship to ecosystem functioning: a review. Biodivers Conserv 7:1221–1244.

Fontcuberta A, Kapun M, Tran Van P, Purcell J, Chapuisat M (2022) Effects of social organization and elevation on spatial genetic structure in a montane ant. Ecol Evol 12:e8813.

Gaston KJ (2009) Geographic range limits of species. P Roy Soc B-Biol Sci 276:1391–1393.

Gotwald WH (1986) The beneficial economic role of ants. Economic Impact and Control of Social Insects, 290–313.

Guenard B, Weiser MD, Gomez K, Narula N, Economo EP (2017) The Global Ant Biodiversity Informatics (GABI) database: synthesizing data on the geographic distribution of ant species (Hymenoptera: Formicidae). Myrmecol News 24:83-89.

Holec M, Frouz J (2006) The effect of two ant species *Lasius niger* and *Lasius flavus* on soil properties in two contrasting habitats. Eur J Soil Biol 42:S213–S217.

Hölldobler B, Wilson EO (1990) The Ants. Harvard University Press.

Hunt GJ, Gadau JR (2016) Editorial: Advances in genomics and epigenomics of social insects. Fron Gen 7:199.

Janicki J, Narula N, Ziegler M, Guénard B, Economo EP (2016) Visualizing and interacting with large-volume biodiversity data using client–server web-mapping applications: The design and implementation of antmaps.org. Ecol Inform 32:185–193.

Jones CG, Lawton JH, Shachak M (1994) Organisms as ecosystem engineers. In Ecosystem management (pp. 130–147). Springer.

Mandel RD, Sorenson CJ (1982) The role of the western harvester ant (*Pogonomyrmex occidentalis*) in soil formation. Soil Sci Soc Am J 46:785–788.

McGuire D, Sankovitz M, Purcell J (2022) A novel distribution of supergene genotypes is present in the socially polymorphic ant *Formica neoclara*. BMC Ecol Evol 22:47.

Minter NJ, Franks NR, Brown KAR (2012) Morphogenesis of an extended phenotype: four-dimensional ant nest architecture. J R Soc Interface 9:586–595.

Paton TR, Humphreys GS, Mitchell PB (1995) Soils: A New Global View. Yale University Press.

Petal J (1978) Role of ants in ecosystems. International Biological Programme.

Pierce D, Sun P, Purcell J, Brelsford A (2022) A socially polymorphic *Formica* ant species exhibits a novel distribution of social supergene genotypes. J Evolution Biol 35:1031–1044.

Purcell J, Lagunas-Robles G, Rabeling C, Borowiec ML, Brelsford A (2021) The maintenance of polymorphism in an ancient social supergene. Mol Ecol 30:6246–6258.

Reichman OJ, Seabloom EW (2002a) The role of pocket gophers as subterranean ecosystem engineers. Trends Ecol Evol 17:44–49.

Reichman OJ, Seabloom EW (2002b) Ecosystem engineering: a trivialized concept?: Response from Reichman and Seabloom. Trends Ecol Evol 17:308.

Salem MZ, Hole FD (1968) Ant (*Formica exsectoides*) Pedoturbation in a forest soil. Soil Sci Soc Am J 32:563–567.

Schultz TR (2000) In search of ant ancestors. P Natl Acad Sci USA 97:14028–14029.

Spicer JI, Gaston KJ (1999) Amphipod gigantism dictated by oxygen availability? Ecol Lett 2:397-403.

Stoks R, Govaert L, Pauwels K, Jansen B, De Meester L (2016) Resurrecting complexity: the interplay of plasticity and rapid evolution in the multiple trait response to strong changes in predation pressure in the water flea *Daphnia magna*. Ecol Lett 19:180-190.

Thomas FIM, Cornelisen CD, Zande JM (2000) Effects of water velocity and canopy morphology on ammonium uptake by seagrass communities. Ecology 81:2704–2713.

Wilby A (2002) Ecosystem engineering: a trivialized concept? Trends Ecol Evol 17:307.

Wright JP, Jones CG (2004) Predicting effects of ecosystem engineers on patch-scale species richness from primary productivity. Ecology 85:2071–2081.

Chapter I: Ant nests differentially affect soil chemistry across elevational gradients

Introduction

Ants are characterized as ecosystem engineers (Jones et al. 1994; Folgarait 1998). Subterranean ant species alter soil hydrology and nutrients by excavating underground nests, thus modulating flows of limiting resources and increasing the complexity of the subterranean habitat (Crist and Wiens 1996). They increase soil drainage and aeration through the formation of underground tunnels (Alvarado et al. 1981) and incorporate nutrients such as carbon and nitrogen into soil through food storage (Friese and Allen 1993), aphid cultivation (Folgarait 1998), and the accumulation of feces and corpses (Gay 1993). For example, the effect of harvester ants on vegetation is likely influenced more by their creation of soil nests than by their role as seed harvesters (Wilby et al. 2001). Indeed, the local effects of active ant colonies on soils are well known (Nkem et al. 2000; Moutinho et al. 2003; Sankovitz and Breed 2019).

Although soil bioturbation by ants is a fine-scale disturbance, it induces landscape-level changes by allowing infiltration (Eldridge 1994; Whitford 2000) and redistribution of nutrients (Nkem et al. 2000). The functional significance of these structures is likely to vary, and the same type of soil disturbance may produce variable effects in different habitats (Steinberger and Whitford 1984; Whitford and DiMarco 1995; Snyder et al. 2002). Few studies, however, have considered the effects of ant-created structures across different habitats (though see Whitford and DiMarco 1995; Snyder et al. 2002; James et al. 2008; Farji-Brener and Werenkraut 2017), even though

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landscape-scale spatial variability in climate, disturbance, and resource distribution is an essential determinant of many ecosystem processes (Peters and Havstad 2006).

Here, I focus on scaling up from local to landscape-scale soil effects in ant species that span environmental gradients. I investigated how two ant species (*Formica francoeuri* (Bolton 1995) and *Formica sibylla* (Wheeler 1913)) affect both soil moisture and soil chemical properties in mountain ranges of California. Both species build inconspicuous subterranean nests. I conducted this study across two elevational gradients of ~1000 m in the Sierra Nevada and San Jacinto Mountain ranges.

The aim of the present study was to investigate two questions: *Do soil moisture and levels of carbon and nitrogen* (i) *differ consistently between ant nest soils compared with adjacent non-nest soils, irrespective of ant species or habitat?* and (ii) *change predictably with the abiotic environment along an elevational gradient?* Per our knowledge of ants as ecosystem engineers, I predicted that soil properties would be significantly different in nests compared to control soil. Specifically, I expected to observe an enrichment of carbon and nitrogen in nest relative to control samples. Further, since many factors that influence soil quality and ant behavior - such as temperature (Sankovitz and Purcell 2021), precipitation (Krushelnycky et al. 2005), soil type (Kumar and O'Donnell 2009), and vegetation (Wagner and Fleur Nicklen 2010) - vary with elevation, I predicted that the impact of ants on soil properties would vary with elevation. However, I did not have an *a priori* expectation about the orientation of this relationship.

Materials and methods

Study area and sampling methods

I conducted this study in June-July 2018 within the San Jacinto Mountains and the Sierra Nevada, California, USA. I sampled soil and workers from 32 *F. francoeuri* colonies across eight sites along two transects (on north- and south-facing slopes) in the San Jacinto Mountains (spanning 371-1370 m) and 41 *F. sibylla* colonies across nine sites along two transects (on east- and west-facing slopes) in the Sierra Nevada (spanning 1776-2350 m) (Table S1.1). These two species are common within their respective mountain ranges, but they are not found in sympatry. Since many *Formica* species look similar, confirming species identification based solely on morphology can be challenging. Therefore, I sequenced 1-2 ant workers per colony for genetic species identification (see 'Species Confirmation' section in supplementary materials for detailed methods and results).

At each nest, I sampled soil from both the nest itself and a control area approximately 10 m away, in a direction chosen with a random number generator. I took samples from a depth of approximately 10 cm (Nkem et al. 2000; Wagner et al. 2004) using a trowel (samples were approximately 10 x 10 cm³, spanning 10-20 cm deep). Additionally, to investigate how ant nest architecture may affect soil properties in contrasting environments, I revisited one low and one high elevation site in the San Jacinto Mountains and took soil cores 35 cm deep (three nest cores and one control core per site). I sampled soil from four depths in each core.

Measurement of soil moisture and chemical properties

I sieved (2mm) each soil sample to exclude rocks and sticks and divided the samples into halves. Using one half, I immediately measured the wet weight and then dried the samples in an oven at 105 °C for 24 h. To determine moisture content, I then measured dry weight, calculated the difference between the two weights, and divided the difference by the dry weight to standardize by volume. I dried the other half sample at 55 °C overnight, then thoroughly ground and passed it through a 100-mesh sieve. I homogenized the soil and packaged ~80 mg of each sample into a tin capsule. Samples were analyzed for total carbon and nitrogen content at the UCR Environmental Sciences Research Laboratory using a Flash EA1112 elemental analyzer.

Statistical analyses

I performed all statistical analyses in R (V 5.3.5, The R Foundation for Statistical Computing). I used the *lmer* function from the *lme4* package (Bates et al. 2014) and the *lmerTest* function from the *lmerTest* package (Kuznetsova et al. 2017) to build a linear mixed-effects model for each species in order to compare the nest and control soil across elevation. I built two separate models because each ant species is found in only one mountain range, so I cannot distinguish between range-specific and species-specific differences in my analysis. In these models, soil C, N, and moisture were response variables, sample location (nest or control), elevation, and the interaction between the two were fixed effects, and nest ID and transect were random effects. To examine if there was an effect of transect, I used a likelihood ratio test to compare models with and

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without transect as a random effect. Additionally, I ran linear mixed effect models to examine whether baseline soil properties vary with elevation in the control samples from each mountain range. In these models, soil C, N, and moisture were response variables, elevation was a fixed effect, and transect was a random effect. To examine the variance of carbon and nitrogen at various depths throughout the nest, I used linear models to analyze the results of the 35 cm nest soil cores. In these models, soil sample depth was the independent variable, and the percentage of carbon or nitrogen was the response variable.

Results and discussion

The difference in soil carbon and nitrogen content between ant nests and control sites varied with elevation. The extent to which control soil was enriched with nitrogen was significantly positively correlated with elevation (and the climatic factors that covary with elevation (Table S1.2)) across both mountain ranges, and the extent to which control soil was enriched with carbon was significantly positively correlated with elevation in the Sierra Nevada (Table S1.3). At higher elevations, nest soil had higher amounts of carbon and nitrogen (Figure 1.1; Table 1.1) than adjacent control soil samples. This pattern was consistent with my expectations. However, at lower elevations, nest soil tended to have reduced carbon and nitrogen relative to controls (Table 1.1), which seemed counter-intuitive. The unexpected pattern of nutrient depletion in low elevation ant nests led us to consider possible limitations of studies investigating soil properties in ant nests. While moisture of control soil significantly increased with elevation in the Sierra Nevada, there

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was no significant pattern along the elevational gradient of the San Jacinto Mountains

(Table S1.3). I observed no significant pattern of soil moisture in ant nests compared with

controls (Table 1.1). Here, I describe and interpret the patterns that I found in my study,

and then I consider potential sources of noise in my sampling approach and suggest steps

that could be taken to reduce variance not associated with the biological research

questions in future studies.
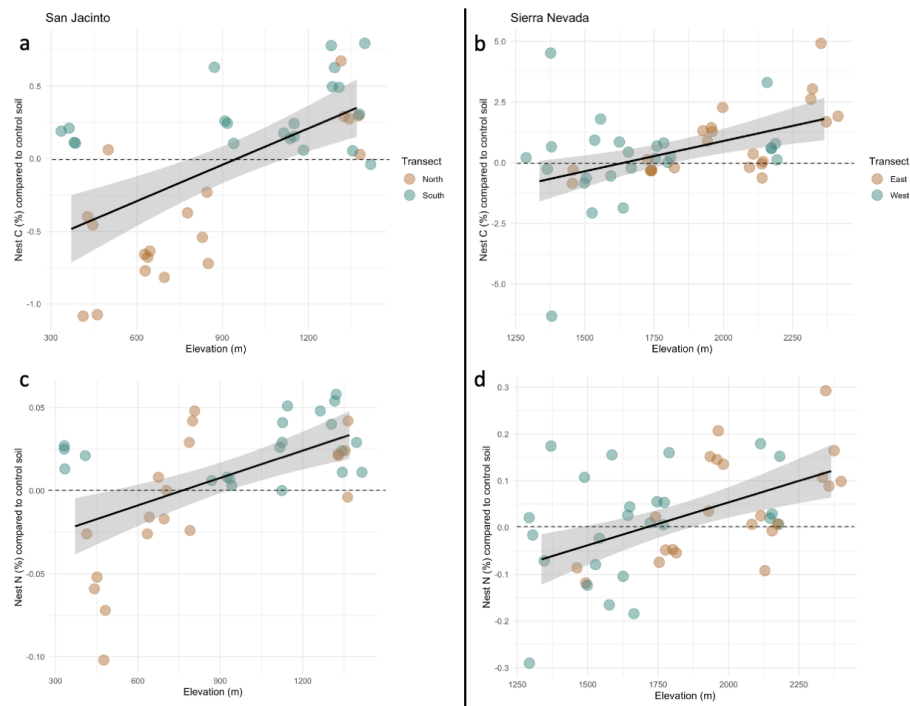
Fig. 1.1 Percentage of carbon and nitrogen in nest soil compared to control soil. At lower elevations, nest soil had lower amounts of carbon and nitrogen than control soil, but at higher elevations, nest soil had higher amounts of carbon and N. Panels (a) and (c) show *F. francoeuri*, while (b) and (d) show *F. sibylla. The dotted line represents the baseline amount of carbon or nitrogen in the (control) soil.*

Table 1.1. Linear mixed-effects model results. The extent to which nest soil was enriched with carbon and nitrogen was significantly correlated with elevation across both mountain ranges.

Mountain range	Soil property	Variable	DF	F	P
San Jacinto	C	Treatment or control	1,30	8.5180	0.006608**
		Elevation (m)	1,29.277	0.2959	0.590603
		Treatment or control x Elevation (m)	1,30	11.0332	0.002362**
	N	Treatment or control	1,30	5.8779	0.021567*
		Elevation (m)	1,30	0.7561	0.391446
		Treatment or control x Elevation (m)	1,30	10.3889	0.003051**
	Moisture	Treatment or control	1,30.002	0.2455	0.6239
		Elevation (m)	1,29.999	0.3651	0.5502
		Treatment or control x Elevation (m)	1,30.002	0.2482	0.6220
Sierra Nevada	C	Treatment or control	1,39	6.7280	0.01330*
		Elevation (m)	1,39	5.3051	0.02668*
		Treatment or control x Elevation (m)	1,39	9.2211	0.00425**
	N	Treatment or control	1,38.999	8.5375	0.005761**
		Elevation (m)	1,1.612	1.9928	0.320231
		Treatment or control x Elevation (m)	1,38.999	11.3626	0.001701**
	Moisture	Treatment or control	1,39	0.0466	0.830159
		Elevation (m)	1,38.387	9.3748	0.004005**
		Treatment or control x Elevation (m)	1,39	0.0648	0.800466

Intriguingly, data from both ranges showed a trend of low elevation nests containing soil depleted in carbon and nitrogen compared to control soils, whereas high elevation nest soils were enriched in carbon and nitrogen compared to control soils. While these consistent trends emerged, the magnitude of ant nest effects on soil varied between the mountain ranges and transects within range. The relative increase or decrease in carbon and nitrogen levels in ant nest soil compared to control soil was up to an order of magnitude greater in the *F. sibylla* nests in the Sierra Nevada compared to the *F. francoeuri* nests in the San Jacinto mountains. Thus, my results highlight the complexity of generalizing the effects of ant nests on soil properties across elevations and landscapes that may vary in water drainage, soil type, and climate.

I was unsurprised to find higher soil carbon and nitrogen in nest soil compared with control soil in many sites. This result is consistent with studies that have found that

Formica nests contain higher amounts of nutrients and organic matter (Kristiansen and Amelung 2001; Drager et al. 2016), which suggests a large community of soil microbes in nest soils that would encourage greater rates of decomposition and nitrogen mineralization (as has been found in harvester ants (Wagner and Jones 2006)). I also expected differences in the effects of nests across elevation gradients, as landscape-level processes affect the distribution of soil nutrients. My results align with studies that have found a differential effect of ants on soil processes and function across various climates and land-use regimes (Folgarait 1998).

The result that nest soils showed nutrient depletion compared to control soil in many lower-elevation sites was more surprising. I identify three alternative, biologically relevant hypotheses that may explain this counterintuitive finding. The pattern could result from soil texture and permeability; generally, more sandy soil is nutrient-poor because nutrients tend to leach through (e.g. Ge et al. 2019). Observationally, higher-elevation sites appeared to have more compact soil, which may limit the depth of nests and resulting nutrient accumulation. Conversely, ants may excavate deeper in less-compact soil, bringing vegetation and prey items well below the soil surface and bringing nutrient-poor soil to the surface. My complementary research revealed that ant nest depth depends on surface temperature in lab-based groups of *Formica podzolica* workers (Sankovitz and Purcell 2021). Hence, nest architecture may vary consistently with elevation, causing standard soil sampling approaches, like the one used here, to yield samples from different portions of nests at different points along elevation gradients. To investigate the possibility that my standardized sampling approach systematically

collected soil from different portions of nests across sites, I revisited one low and one high elevation site in the San Jacinto Mountains and took soil cores 35 cm deep. However, I found no correlation of carbon or nitrogen with soil depth in these preliminary samples (Table S1.4).

Several issues with my sampling protocol could yield significant differences in soil metrics that are not mediated by biologically interesting effects of ant nests on soil and should be adjusted in future studies. First, even if thoroughly homogenized, the quantity of soil used by the elemental analyzer is minuscule - mere grams - making obtaining a representative sample difficult. In this study, I took measurements from a single soil sample within the nest and a second one near the nest (nest and control sample, respectively). Comparing multiple nest and control samples for each nest could provide a more representative measure. Second, ant nests complicate this process further because the bioturbation and addition of organic matter by ants, in addition to variation in nest architecture, create a plot of soil that is highly variable in all three dimensions. I took samples from a standard 10 cm below the soil surface, following established protocols (Nkem et al. 2000; Wagner et al. 2004), but my concurrent study indicated that nest depth could strongly covary with elevation and temperature (Sankovitz and Purcell 2021). Hence, I may have breached the nest in some samples and not others, with a bias associated with the average temperature of each locality. This sampling approach might be accurate for ants that excavate superficial nests, like Argentine ants, but both sampling soil cores and investigating the depth of the highest nest chambers may be necessary to ensure that equivalent samples are compared in ant species that dig deeper nests. Due to

these potential limitations, the results presented here should be considered intriguing patterns that hint at ecologically significant processes at play but should be investigated further with sampling encompassing a broader spectrum of possible nest architecture.

Much research has focused on the effects of ants on ecosystem processes within single populations (De Bruyn and Conacher 1990; MacMahon et al. 2000; Decaëns et al. 2002; Folgarait et al. 2002), but few studies have examined how these effects vary across environmental gradients. Both my original samples and the follow-up samples taken using a soil core contained significant variability, demonstrating the difficulty of taking a representative soil sample inside ant nests. Despite my efforts to homogenize soil samples, analyzing mere grams of soil from an entire nest probably does not capture a representative profile of nest soil. I recommend that future research on ant soil interactions sample soil from multiple locations within an ant nest, both horizontally and vertically in the soil profile. Overall, my results suggest that the magnitude of ant nest effects on nutrients is likely influenced by factors that vary with elevation, like climate and soil type. Future research on ant ecosystem engineering should work to generalize how ants affect soil properties in distinct geological and climatic zones.

References

Alvarado A, Berish CW, Peralta, F (1981) Leaf-cutter ant (*Atta cephalotes*) influence on the morphology of andepts in Costa Rica. Soil Sci Soc Am J 45:790-794.

Bates D, Mächler M, Bolker B, Walker S (2014) Fitting linear mixed-effects models using lme4. arXiv [stat.CO]

Crist TO, Wiens JA (1996) The distribution of ant colonies in a semiarid landscape: implications for community and ecosystem processes. Oikos 76:301–311.

De Bruyn LAL, Conacher AJ (1990) The role of termites and ants in soil modification-a review. Soil Res 28:55-93.

Decaëns T, Asakawa N, Galvis JH, et al (2002) Surface activity of soil ecosystem engineers and soil structure in contrasted land use systems of Colombia. Eur J Soil Biol 38:267–271.

Drager KI, Hirmas DR, Hasiotis ST (2016) Effects of ant (*Formica subsericea*) nests on physical and hydrological properties of a fine-textured soil. Soil Sci Soc Am J 80:364–375.

Eldridge DJ (1994) Nests of ants and termites influence infiltration in a semi-arid woodland. Pedobiologia 38:481–492.

Farji-Brener AG, Werenkraut V (2017) The effects of ant nests on soil fertility and plant performance: a meta-analysis. J Anim Ecol 86:866–877.

Folgarait PJ (1998) Ant biodiversity and its relationship to ecosystem functioning: a review. Biodivers Conserv 7:1221–1244.

Folgarait PJ, Perelman S, Gorosito N, et al (2002) Effects of *Camponotus punctulatus* ants on plant community composition and soil properties across land-use histories. Plant Ecol 163:1–13.

Friese CF, Allen MF (1993) The interaction of harvester ants and vesicular-arbuscular mycorrhizal fungi in a patchy semi-arid environment: the effects of mound structure on fungal dispersion and establishment. Funct Ecol 13-20.

Gay H (1993) Animal-fed plants: an investigation into the uptake of ant-derived nutrients by the far-eastern epiphytic fern *Lecanopteris* Reinw. (Polypodiaceae). Biol J Linn Soc 50:221-233.

Ge N, Wei X, Wang X, et al (2019) Soil texture determines the distribution of aggregate-associated carbon, nitrogen and phosphorous under two contrasting land use types in the Loess Plateau. Catena 172:148–157.

James AI, Eldridge DJ, Koen TB, Whitford WG (2008) Landscape position moderates how ant nests affect hydrology and soil chemistry across a Chihuahuan Desert watershed. Landsc Ecol 23:961-975.

Jones CG, Lawton JH, Shachak M (1994) Organisms as ecosystem engineers. In: Ecosystem management. Springer, pp 130–147.

Kristiansen SM, Amelung W (2001) Abandoned anthills of *Formica polyctena* and soil heterogeneity in a temperate deciduous forest: morphology and organic matter composition. Eur J Soil Sci 52:355–363.

Krushelnycky PD, Joe SM, Medeiros AC, Daehler CC, Loope LL (2005) The role of abiotic conditions in shaping the long-term patterns of a high-elevation Argentine ant invasion. Divers Distrib 11:319-331.

Kumar A, O'Donnell S (2009) Elevation and forest clearing effects on foraging differ between surface, and subterranean, foraging army ants (Formicidae: Ecitoninae). J Anim Ecol 91-97.

Kuznetsova A, Brockhoff PB, Christensen RHB (2017) lmerTest package: tests in linear mixed effects models. J Stat Softw 82:1–26.

MacMahon JA, Mull JF, Crist TO (2000) Harvester ants (*Pogonomyrmex* spp.): Their community and ecosystem influences. Annu Rev Ecol Syst 31:265–291.

Moutinho P, Nepstad DC, Davidson EA (2003) Influence of leaf-cutting ant nests on secondary forest growth and soil properties in Amazonia. Ecology 84:1265–1276.

Nkem JN, Lobry de Bruyn LA, Grant CD, Hulugalle NR (2000) The impact of ant bioturbation and foraging activities on surrounding soil properties. Pedobiologia 44:609–621.

Peters DPC, Havstad KM (2006) Nonlinear dynamics in arid and semi-arid systems: Interactions among drivers and processes across scales. J Arid Environ 65:196–206.

Sankovitz MA, Breed MD (2019) Effects of *Formica podzolica* ant colonies on soil moisture, nitrogen, and plant communities near nests. Ecol Entomol 44:71-80.

Sankovitz M, Purcell J (2021) Ant nest architecture is shaped by local adaptation and plastic response to temperature. Sci Rep 11:23053.

Snyder SR, Crist TO, Friese CF (2002) Variability in soil chemistry and arbuscular mycorrhizal fungi in harvester ant nests: the influence of topography, grazing and region. Biol Fertil Soils 35:406–413.

Steinberger Y, Whitford WG (1984) Spatial and temporal relationships of soil microarthropods on a desert watershed. Pedobiologia 26:275–284.

Wagner D, Fleur Nicklen E (2010) Ant nest location, soil nutrients and nutrient uptake by ant-associated plants: does extrafloral nectar attract ant nests and thereby enhance plant nutrition? J Ecology 98:614-624.

Wagner D, Jones JB (2006) The impact of harvester ants on decomposition, N mineralization, litter quality, and the availability of N to plants in the Mojave Desert. Soil Biol Biochem 38:2593–2601.

Wagner D, Jones JB, Gordon DM (2004) Development of harvester ant colonies alters soil chemistry. Soil Biol Biochem 36:797–804.

Whitford WG, DiMarco R (1995) Variability in soils and vegetation associated with harvester ant (*Pogonomyrmex rugosus*) nests on a Chihuahuan Desert watershed. Biol Fertil Soils 20:169–173.

Whitford WG (2000) Keystone arthropods as webmasters in desert ecosystems. Invertebrates as webmasters in ecosystems 25–41.

Wilby A, Shachak M, Boeken B (2001) Integration of ecosystem engineering and trophic effects of herbivores. Oikos 92:436–444.

Chapter II: Ant nest architecture is shaped by local adaptation and plastic response to temperature

Introduction

The nests of social insects have doubtlessly contributed to their widespread ecological success by providing shelter and defensive benefits (Minter et al. 2012; Dawkins 2016). Ants (Formicidae) exhibit particularly diverse nesting strategies, which include nesting in soil, leaf litter, plants, and cavities, allowing them to persist in most terrestrial habitats. Many ant species nest in soil, modifying soil ecosystems in the process (Tschinkel 2015). They increase soil drainage and aeration by forming underground tunnels and chambers and incorporate nutrients into soil through food storage and the accumulation of feces and corpses (Brian and Brian 1978). Hence, the benefits of ant nests are multifaceted, providing advantages to both ecosystem functioning and colony fitness.

Numerous studies measured above-ground aspects of ant nests (De Bruyn and Conacher 1990; Sankovitz et al. 2019), but subterranean nest architecture has received comparatively little research attention, likely due to the difficulties of observing and measuring this belowground aspect of ant life (but see Tschinkel 2003; Pinter-Wollman 2015). The structure, or architecture, of ant nests allows for the precise regulation of some environmental conditions. For example, large colonies of wood ants (*Formica rufa* group) build elaborate and long-lasting thatch mounds constructed of plant materials and mineral soil, reducing moisture loss and regulating temperature for optimal colony growth and performance (Rosengren et al. 1987; Hölldobler and Wilson 1990). Temperature is an important aspect of colony growth and survival (Savolainen and

Vepsäläinen 1988); the centers of these nests are particularly important for providing a favorable microclimate for brood development (Frouz et al. 2016). Nest depth predominantly affects microclimatic conditions faced by colonies with subterranean nests (Seeley and Heinrich 1981) since temperature, humidity, and air composition vary predictably with soil depth (Hillel 1998). Yellow meadow ants (*Lasius flavus*) respond to taller vegetation by building larger 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). Harvester ants (*Pogonomyrmex* spp.) create and maintain vegetation-free zones around their nests by removing debris and clipping the vegetation, reducing transit time for foragers, decreasing fire and predation risk, and increasing exposure to solar radiation (MacMahon et al. 2000). These examples show that nest construction can lead to decreased environmental hazards and enhance conditions for colony development. As Jones et al. (1994) postulated, ants' nest structures are intentional responses to their environmental surroundings and thus an inferred extended plastic phenotypic trait that may allow ants to occupy many different habitats. Therefore, as colonies' needs vary with time and environment, the properties of nests likely also change in response (Jouquet et al. 2006).

In addition to providing environmental stability, ant nest architecture shapes and, in turn, is shaped by collective behavior and therefore provides an opportunity to study individual- and colony-level behaviors in a shared, dynamic environment (Pinter-Wollman 2015). Physiology and individual-level behavioral variation can have colony-level effects reflected in nest architecture; for example, a building pheromone added by

individual workers to the nest material has been shown to be a critical factor that controls

the growth and form of nest architecture (Khuong et al. 2016). Similarly, in yellow

meadow ant colonies, the angular distribution of tunnels is probably a result of local

competition among workers (Minter et al. 2012). Likewise, nest architecture can

influence colony-level behavior; as harvester ant nest chamber connectivity and

redundancy of connections among chambers increase, so does a colony's speed of

recruitment to food (Pinter-Wollman 2015).

Most previous studies on subterranean nest architecture have been conducted in a

single habitat, yet many ant species ranges span distinct habitats and climates, some of

which are rapidly changing with climate and other anthropogenic disturbances (Bishop et

al. 2019). Global warming has stimulated worldwide studies aiming to assess or predict

the impact of rising environmental temperatures on organisms (Sala et al. 2000; Braschler

et al. 2020; Roeder et al. 2021). Many of these studies have focused on thermal tolerances

of terrestrial ectotherms (Deutsch et al. 2008; Diamond et al. 2012; Hoffmann et al. 2013)

because they represent most terrestrial biodiversity (Wilson 1992) and are especially

likely to be vulnerable to climate warming due to the strong influence of environmental

temperature on their physiological and behavioral functions (Huey and Stevenson 1979).

Social insects, including ants, provide a unique opportunity to study how behaviors could

mitigate the impact of warming, as temperature affects the performance of both

individuals and the colony. Since nest architecture affects and reflects the

thermoregulation ability of a colony, it likely plays a vital role in the ability of these

insects to respond to changing climate conditions.

Formica podzolica (Francoeur, 1973) is a montane ant with colonies ranging from 5000 to 100,000 workers. They construct conspicuous soil mound nests, which can exceed 2 m in diameter (Deslippe and Savolainen 1995). Nests occur in pine and aspen stands from Alaska to New Mexico, at altitudes up to approximately 3000 m, so this species is ideal for studying how local adaptation and extrinsic conditions shape nest architecture. Although no published descriptions of complete nests exist to my knowledge, alteration of nest architecture may be a key to these ants' survival in a wide range of environments. In this study, I investigate the extent to which the extended phenotype of nest architecture is either (i) plastic and varies with soil surface temperature or (ii) locally adapted to the population's native climate. Using custom-built nest boxes and temperature chambers, I carried out a laboratory transplant experiment with populations of *F. podzolica* from two different elevations separated by ~1000 m. I measured nest size (depth and area) and complexity (number of tunnels) daily during week-long trials.

Materials and methods

I compared nests excavated by *F. podzolica* workers (collected from a total of 60 mature colonies) from sites within two elevational ranges under two temperature treatments (Figure 2.1). I collected colonies in Boulder County, CO, USA, during June-September of 2019. Lower elevation sites spanned ~400 m and included Platt-Rogers Memorial Park (39.98' N, 105.44' W, ~2,100 m), Mud Lake Open Space (39.98' N, 105.51' W, ~2,500 m), and Reynolds Ranch (40.17' N, 122.24' W, ~2,200 m). Higher elevation sites

spanned ~200 m and included areas surrounding the Sourdough Trail (40.02′ N 105.31′ W, ~3,000 m) and Brainard Lake Recreation Area (40.08' N, 105.57' W, ~3,200 m). Although landscape features are similar across all sites (closed-canopy pine forest with limited undergrowth diversity), daily temperatures vary significantly (see below).
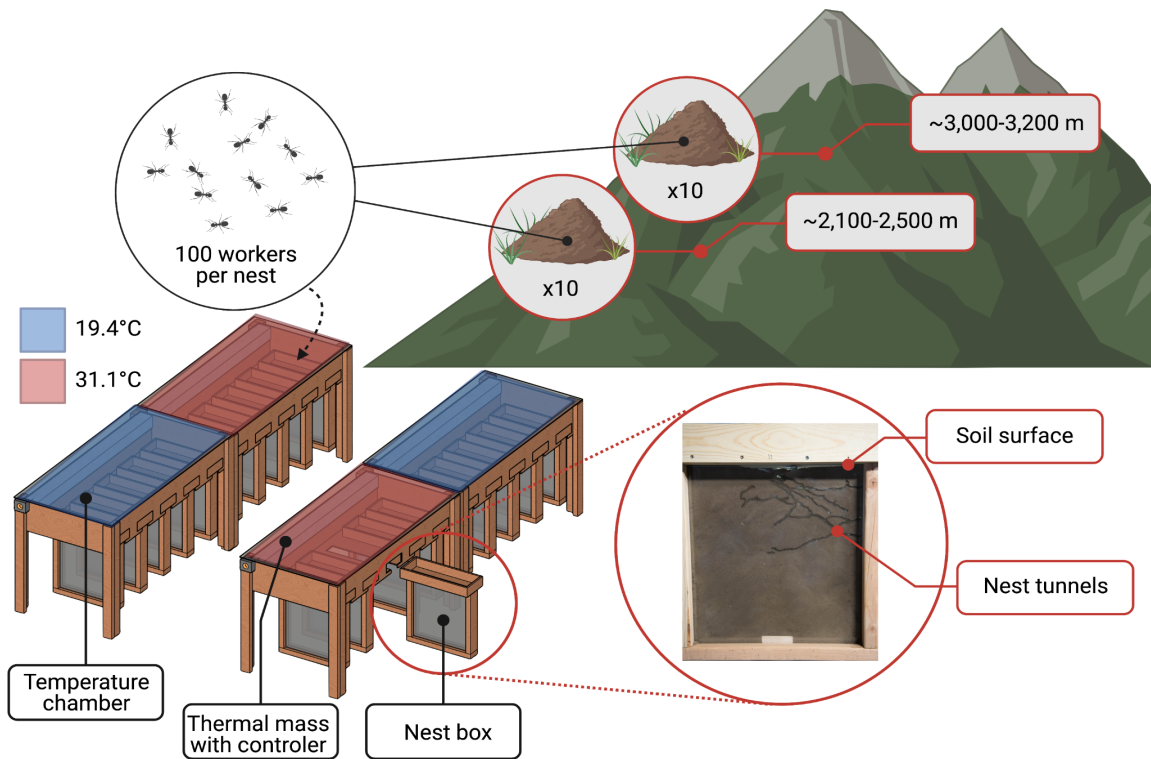
Figure 2.1. Overview of the experimental design. I collected 100 workers from each of 20 colonies per trial (10 from higher elevation sites and 10 from lower elevation sites). I allowed colonies to excavate nests in boxes attached to temperature chambers at the soil surface level. There were four chambers, each containing five nest boxes and a mixture of low- and high-elevation colonies. I repeated this experiment three times during one summer to obtain a total sample of 60 colonies. Created with BioRender.com.

During each of three 1-week trials, I collected 100 workers directly from the nest entrance of each of 20 colonies (10 from low elevation sites and 10 from high elevation sites each time) using forceps. Overall, I collected 20 colonies per trial, for a total of 60 colonies. I sampled nests across all sites for each trial, and colonies were not re-sampled during the experiment. I housed the workers in 20 transparent nest boxes (45.72 x 50.8 x

13.81 cm) in a laboratory setting and allowed them one week to excavate nests. Boxes contained a mixture of soil in equal parts from each site that I sieved (0.635 cm), mixed thoroughly, and compacted at a constant rate across boxes. The wood boxes had glass walls and were kept dark with black fabric to mimic a subterranean habitat. Four temperature chambers (also constructed of wood) encapsulated five boxes each from the soil surface up, with most of each nest box extending below the chambers and in contact with the ambient air temperature of the room. This setup mimics the expected natural temperature gradient, with the soil surface warm during the day and the deeper parts of the nest cooler. Temperature chambers were heated via a thermal mass consisting of a concrete mortar mix surrounding radiant heating tubing connected to a temperature-controlling outlet. I monitored the soil temperature of one box per chamber per trial using iButton temperature sensors at the surface, halfway deep, and bottom of the boxes (Figure S2.1).

I offered ants plain water and sugar water in cotton-plugged Olympus 1.7ml Microtubes, placed at the soil surface inside each box. To test the effects of temperature on nest architecture, I applied a high-temperature treatment to 10 boxes (31.1°C, the July high temperature averaged from 1981-2010 in Nederland, CO at ~2,400 m; National Oceanic and Atmospheric Administration (NOAA) 2019) and a cooler temperature treatment to the other 10 boxes (19.4°C, the July high temperature averaged from 1981-2010 in Ward, CO at ~2,850 m; NOAA 2019). These temperature treatments were taken from NOAA Weather Observation Stations near my low- and high-elevation sites. The ambient air temperature in the room that contained the boxes was kept at 15.5°C. I

exposed half of the low-elevation colony fragments to the high-temperature treatment at the soil surface and the other half to the low-temperature treatment at the soil surface, with the same treatments for the high-elevation colony fragments. I replicated the experiment three times for a total of 60 colonies.

Every day throughout each trial, I took photographs of both sides of every box to measure the following attributes of nest architecture: nest depth, area of soil excavated (measured as the combined tunnel area from both sides of the nest box), and number of tunnels. I defined a tunnel as one branch of uninterrupted excavated soil between nodes, connection points between two or more tunnels. I imported digital photographs into *ImageJ*, calibrated a 0.0121 cm/pixel scale, and took measurements by tracing the length of each nest, counting the number of pixels making up the tunnels in the photographs (using the high contrast area measurement tool), and counting the number of tunnels (Schneider et al. 2012). Nests varied greatly in their shape, ranging from one simple tunnel to complex networks of tunnels (Figure 2.2). Nine colony fragments did not excavate nests during the experiment; these were spread out across the four different treatments and excluded from all analyses (Table S2.2).
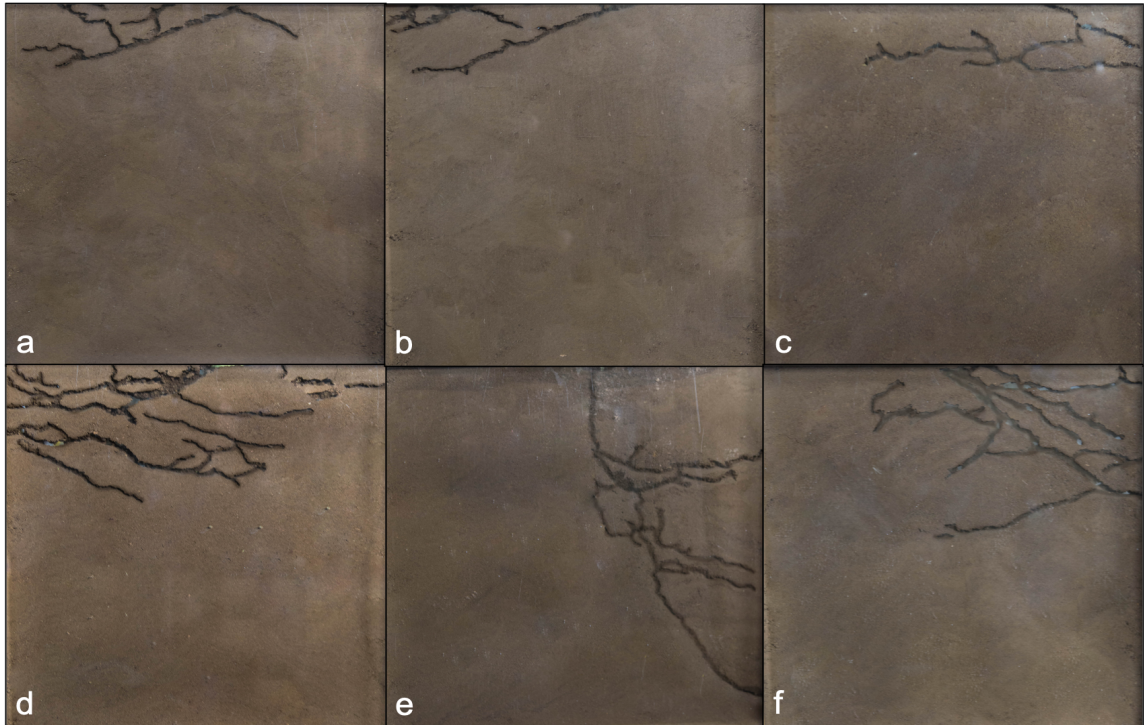
Figure 2.2. Examples of nests after the 7-day excavation period. (a)-(c) are nests from colonies that experienced the low-temperature treatment, whereas (d)-(f) are nests from colonies that experienced the high-temperature treatment.

To determine if the natal environment and temperature treatment influenced nest size and complexity, I compared nests excavated by colony fragments from two natal elevations. In my analysis I used two types of models. First, I used two-way analysis of variance (ANOVA) models with a random effect (R v. 5.3.5, package *stats* (Chambers et al. 1992)) to compare the nest architecture metrics between high- and low-elevation colony fragments tested in high and low temperatures after one week of building. Nest depth, area of soil excavated, and number of tunnels at the end of each one-week trial represented my response variables, colony natal elevation (low or high), temperature treatment, and their interaction were fixed effects, and colony collection site was a

random effect. I confirmed the homogeneity of variance using a Levene's test from the *car* package (Fox 2015).

Second and in complement I used a repeated-measures ANOVA (*stats* package) to compare nest architecture measurements across all seven days of observation to assess whether excavation progressed at the same rate between treatments. I removed observations with a value of 0 (some colony fragments took a day or two to begin excavation). Nest depth, area of soil excavated, and number of tunnels on each day represented my response variables, colony natal elevation, temperature treatment, day, and their interaction were fixed effects, and colony collection site was a random effect. To determine at what day nest depth began to differ between temperature treatments, I compared estimated marginal means of depth per temperature treatment between each day (*emmeans* package) with a Bonferroni correction for multiple comparisons.

Results

Over the seven days of observation, groups of 100 workers produced nests ranging from 4.7 to 23.3 cm in total depth. Nest depth differed significantly between temperature treatments (Table 2.1, Figure 2.3a). The nests excavated by workers experiencing the warmer soil surface temperature treatment (31.1°C) were, on average, 1.5 times deeper (15.5 cm, s.e. 0.5) than those produced by workers experiencing the cooler temperature treatment (19.4°C, 10.4 cm, s.e. 0.91).

Table 2.1. Two-way ANOVA results for Day 7 (fully excavated) nests. Nest depth differed significantly between temperature treatments. There was a significant interaction effect between temperature treatment and natal elevation for both tunnel area and the number of tunnels. Df = 1,47. *Significant, α=0.05.

Response variable	Independent variable	F	P
Nest depth (cm)	Elevation	0.220	0.642
	Temperature	23.353	1.48e-05*
	Elevation x temperature	0.694	0.409
Tunnel area (cm^2)	Elevation	1.943	0.1699
	Temperature	0.037	0.8476
	Elevation x temperature	6.473	0.0143*
Number of tunnels	Elevation	2.289	0.1370
	Temperature	0.114	0.7375
	Elevation x temperature	5.157	0.0278*
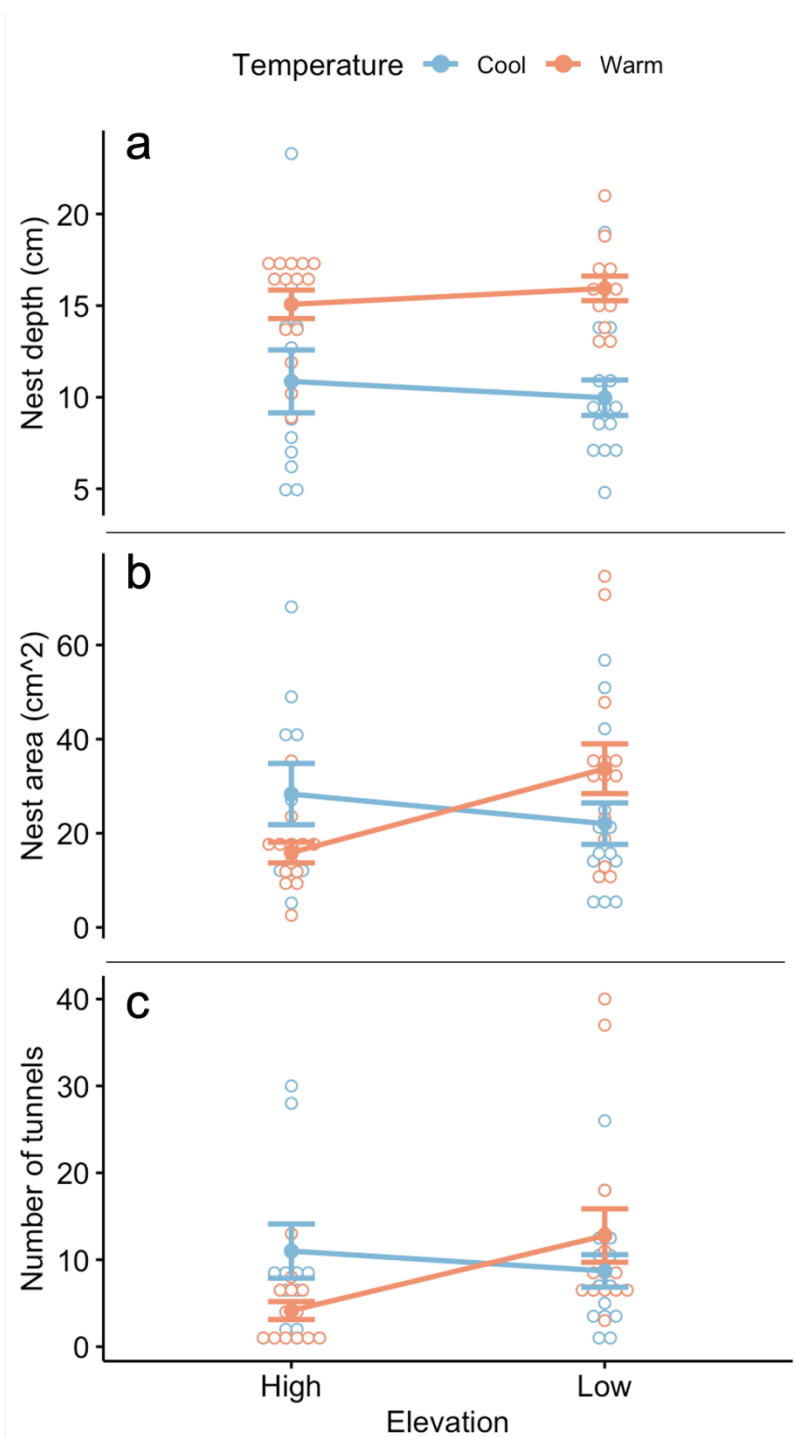
Figure 2.3. Nest depth (a), area of soil excavated (b), and number of tunnels (c) excavated by low- and high-elevation workers under cool (blue) and warm (red) temperature treatments. Each dot represents one observation, and error bars show standard error. Workers experiencing the warm treatment excavated significantly deeper nests than those under the cool treatment, while the tunnel area and the number of tunnels depended on the interaction between the workers' native elevation and the temperature treatment.

The area of the soil excavated ranged between 2.6 and 74.6 cm^2 and did not differ significantly among temperature treatments or natal elevation (Table 2.1; Figure 2.3b). However, there was a significant interaction effect between temperature treatment and natal elevation (Table 2.1). Workers from low elevations excavated tunnels with 2.1 times more area (33.7 cm^2, s.e. 5.3) than those from high elevations (15.9 cm^2, s.e. 2.2) while experiencing the warm temperature treatment. Conversely, workers from high elevations excavated tunnels with 1.5 times more area (28.3 cm^2, s.e. 6.5) than those from low elevations while experiencing the cool temperature treatment (22.0 cm^2, s.e. 4.4).

Nests contained between 1 and 31 tunnels. The effect of temperature treatment on the number of tunnels was not statistically significant, nor was the effect of natal elevation (Table 2.1; Figure 2.3c). However, there was a significant interaction effect (Table 2.1). On average, nests built by workers from low elevations had 3.1 times more tunnels (12.8 tunnels, s.e. 3.1) than those produced by workers from high elevations while experiencing the warm temperature treatment (4.2 tunnels, s.e. 1.0). Conversely, nests built by workers from high elevations had, on average, 1.3 times more tunnels (11 tunnels, s.e. 3.1) than those built by workers from low elevations while experiencing the cool temperature treatment (8.7 tunnels, s.e. 1.9). These results provide further evidence that colonies' response to soil surface air temperature is dependent on their natal environments. There was no standard branching pattern across treatments and depths; instead, tunnel arrangements and individual tunnel length and angle appeared highly variable across colonies.

Excavation did not progress at the same rate across all seven days of the experiment (Figure 2.4). The ants almost universally followed a pattern of making much progress on nest depth at the beginning of the experiment and then decreasing the rate of depth change as they excavated with each subsequent day (Table S2.1). Conversely, nest area and number of tunnels increased relatively steadily throughout the experiment, although at different rates depending on the colony's native elevation and temperature treatment. The repeated measures ANOVA revealed that, for nest depth, the interaction of day and temperature is significant, indicating that nests are getting deeper over time but at different rates depending on temperature treatment (Table 2.2). Both nest area and the number of tunnels increased over time, but the rate did not differ significantly depending on natal elevation or treatment. Nest depth began to significantly differ between temperature treatments on Day 4, and this continued through Day 7 (Table 2.3).
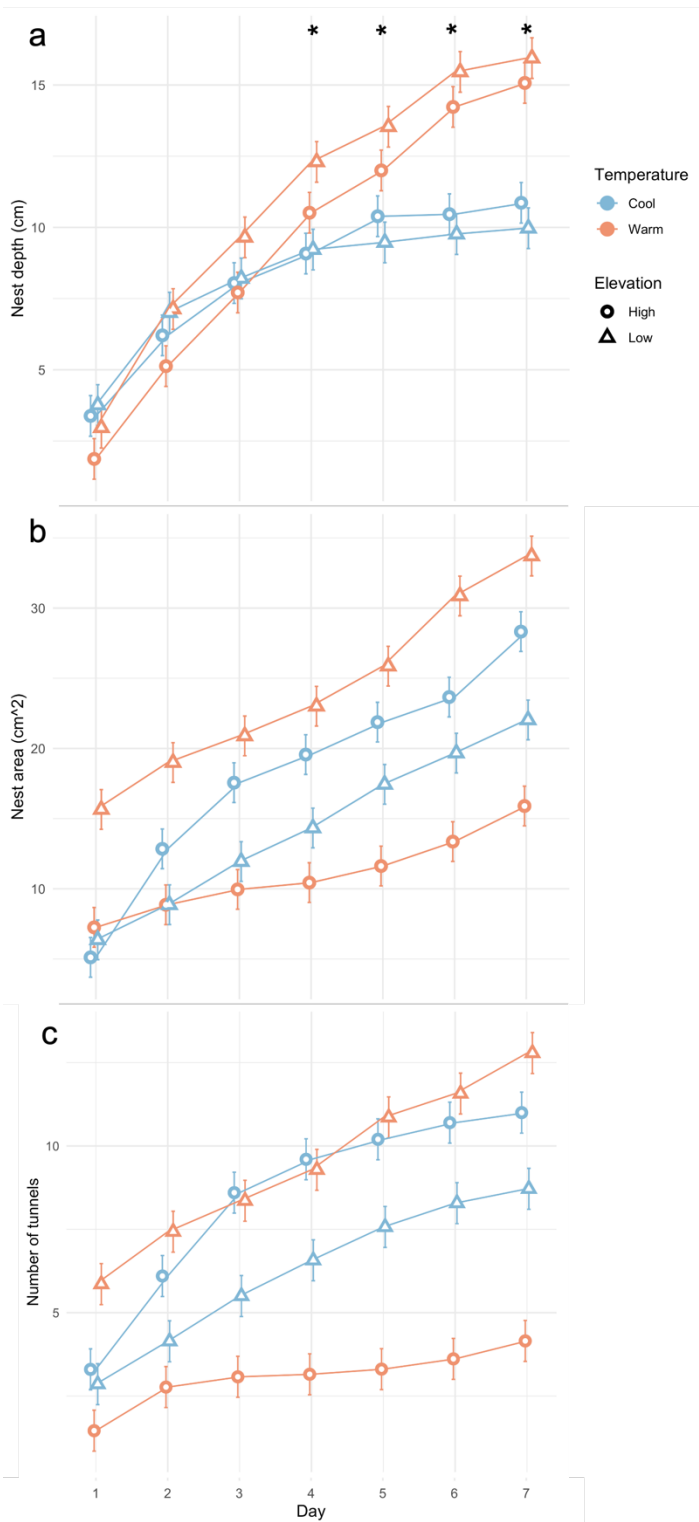
Figure 2.4. Excavation progress in terms of mean nest depth (a), total area (b), and number of tunnels (c) throughout the 7-day trials. Error bars represent standard error. * = significant difference between temperature treatments.

Table 2.2. Repeated measures ANOVA results for all seven days of the experiment. The interaction of day and temperature is significant for nest depth. *Significant, α=0.05.

Response variable	Independent variable	F	P
Nest depth (cm) df = 6,264	Day	70.448	< 2e-16*
	Day x elevation	1.487	0.183
	Day x temperature	9.046	5.33e-09*
	Day x elevation x temperature	0.086	0.998
Tunnel area (cm^2) df = 6,272	Day	27.378	<2e-16*
	Day x elevation	0.618	0.716
	Day x temperature	0.856	0.528
	Day x elevation x temperature	1.865	0.087
Number of tunnels df = 6,272	Day	14.766	1.95e-15*
	Day x elevation	0.757	0.604
	Day x temperature	0.320	0.927
	Day x elevation x temperature	1.232	0.288

Table 2.3. Estimated marginal means, comparing nest depth under cool and warm temperature treatments. Nest depth began to significantly differ between temperature treatments on Day 4. Results are averaged over natal elevation. *Significant, α=0.05.

Day	Estimate	SE	Df	T-ratio	P
1	1.176	1.43	95.7	0.820	0.4141
2	0.233	1.33	73.5	0.175	0.8616
3	1.266	1.33	73.5	0.950	0.3452
4	2.966	1.33	73.5	2.225	0.0291*
5	3.546	1.33	73.5	2.660	0.0096*
6	5.440	1.33	73.5	4.081	0.0001*
7	5.799	1.33	73.5	4.351	< 0.0001*

Discussion

In this study, I observed both a plastic response of nest architecture to conditions experienced during nest construction and evidence of local adaptation to differing conditions in *F. podzolica*. Overall, workers experiencing a high soil surface temperature excavated deeper nests than those experiencing a cooler surface temperature. Neither natal elevation nor temperature treatment alone had a significant effect on the area of soil excavated or the number of tunnels. However, groups collected from lower elevation built larger nests with more tunnels when experiencing warm surface temperatures, while those collected from higher elevation built larger nests with more tunnels when

experiencing cool surface temperatures, suggesting that they perform best in conditions more closely matching their natal habitat.

These results add to a growing collection of evidence that abiotic factors such as weather, soil temperature, moisture (Mikheyev and Tschinkel 2004), and the presence of planes between layers of sediment (ants may excavate horizontal tunnels along such planes) (Minter et al. 2012) affect nest structure, an aspect of the extended phenotype of the colony that continually changes as the colony grows. Ant species ranges can span large elevational and latitudinal gradients, and many species are perennial. Owing to the landscape-scale climatic differences across a species' range, nests built in different localities may differ markedly in form. Although there is evidence of a correlation between soil surface temperature and ambient temperature, respiration, and metabolic rates, further study is needed to determine why soil surface temperature influences nest-building behaviors in *F. podzolica* (Coppernoll-Houston and Potter 2018; Jílková et al. 2015; Kadochová et al. 2017). However, one possibility is that nest depth is a response based on the colony's thermoregulatory needs. Inhabiting deeper nests may allow for the avoidance of higher temperatures at the surface (Bollazzi et al. 2008). This explanation aligns with my observation that the rate of nest depth excavation decreased over the week of the experiment. Ants appeared to eventually settle on a suitable nest depth and focus on excavating larger and more complex nests. Colony fragments experiencing the cool temperature treatment reached this equilibrium faster (around Day 4) than those experiencing the warm temperature treatment, which did not appear to reach an equilibrium. Reducing exposure to surface temperatures by excavating a deeper nest

could increase colony longevity because brood development may be optimized at species-specific temperature preferences (Porter 1988; Stockan and Robinson 2016), although the ideal temperature for brood development remains unknown for *F. podzolica* (Bollazzi et al. 2008). Conversely, for colonies in cool habitats, building shallow nests may increase the temperature inside the nest chambers toward the species optimum.

Other variables such as soil moisture are also expected to influence the determination of nest depth. Nest depth in the leaf-cutter ant *Acromyrmex landolti*, as an example, has been shown to be negatively correlated with soil moisture (Lapointe et al. 1998), and colonies are known to move fungus gardens vertically through the soil profile in search of more optimal soil moisture. However, soil temperature (both surface and subterranean) generally seems to be a more powerful predictor of nesting habits. For instance, *Acromyrmex crassispinus* colonies build deep subterranean nests in the hot soils of Paraguay, yet superficial ones in the colder thermic soils of central Argentina and southern Brazil (Bollazzi et al. 2008; Fowler 2008), although both regions present the same soil moisture regime. I am confident that soil moisture was not a factor driving my results because I standardized it in my experiment. Plasticity in nesting habits based on temperature-sensitive digging may have contributed to the colonization of different habitats worldwide (Hansell and Hansell 2005). The ability of ants to access different microclimates within the full vertical topsoil profile would reduce the constraints of unfavorable soil-surface conditions. Additionally, innovations in building behavior that give rise to the invasion of new habitats might subsequently facilitate adaptive radiation (Hansell and Hansell 2005).

The significant interaction effect of natal elevation and temperature treatment on nest size (area of soil excavated) and complexity (number of tunnels) suggests a level of local adaptation in this extended phenotypic trait. In other words, the elevation that workers originated from did not predict the size or complexity of their resulting nest, but ants excavated larger and more complex nests under conditions that were like their native habitat. While workers from high elevations created larger (greater area) and more complex (more tunnels) nests under the cooler temperature treatment, workers from low elevations created larger and more complex nests under the warmer temperature treatment. This result suggests that metabolic rate may be a locally adapted trait in *F. podzolica* that influences their elevational distribution. The metabolic cold adaptation hypothesis posits that cold environments (e.g. high elevations and latitudes) select for high metabolic rates, even after controlling for body size differences, and that this enables high activity levels when an organism is near its lower thermal limits (Shik et al. 2019). Although I did not expose the ants in my experiment to temperatures near their thermal limits, a locally adapted metabolic rate may still be at play here, leading to higher excavation performance in temperatures more closely matching their original habitat.

Our results suggest that I need to consider extended phenotypes to predict how some ectothermic species respond to climate change. Organisms may be able to behaviorally modulate their exposure to extreme temperatures by creating appropriate thermal microenvironments. I hypothesize that *F. podzolica* workers produce nest architecture that helps them to buffer against thermal extremes. The apparent plasticity of nest depth raises intriguing questions about the extent to which colony life may relax

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selection on worker-level metabolic traits related to climate variability. Workers' thermal performance traits shape ant ecology and distributions at local (Talbot 1934; Cerda et al. 1998; Kaspari et al. 2015) and biogeographic scales (Diamond et al. 2012; Arnan and Blüthgen 2015; Arnan et al. 2015). However, colony-level performance is also governed by a capacity for thermoregulation (Baudier and O'Donnell 2016) since colonies can use nest architecture to thermally manipulate larval development rates (Penick et al. 2017) and shift colony growth rates (Penick et al. 2017).

Behavioral plasticity may help some species mitigate the adverse impacts of climate change and thus should be an essential predictor of an organism's climate warming vulnerability and extinction risk (Kearney et al. 2009). At a small spatial scale, elevation is the dominant factor affecting differences in mean annual surface air temperature. I expect that this altitudinal variability in climatic conditions selects for more plastic phenotypes than species in less climatically variable environments (such as tropical regions at lower latitudes and altitudes) (Ghalambor et al. 2006). In this sense, species inhabiting large altitudinal and latitudinal gradients, like *F. podzolica*, should be excellent models for future studies on the plasticity for behavioral thermoregulation under different climate change scenarios. Further, my findings suggest that nest architecture may be a plastic extended phenotypic trait in other ant species.

Conclusions

Subterranean-nesting ants contribute to critical ecosystem services by building nests. These nests serve numerous essential functions for the colonies living within and alter

soil ecosystems through aeration and the addition of nutrients. In this study, I used a laboratory approach to analyze how natal elevation and surface air temperature influence variation in nest architecture. Worker groups experiencing a warmer temperature produced deeper nests irrespective of their natal population, demonstrating that nest architecture can be a plastic response to the environment. Ants originally from high elevations excavated larger and more complex nests in a cooler temperature, while ants originally from low elevations excavated larger and more complex nests in a warmer temperature. This result suggests a level of local adaptation at play in nest architectural outcomes. My findings suggest that a combination of plasticity and local adaptation of nest architecture contribute to the widespread intraspecific geographic success of *F. podzolica* and likely other ant species.

References

Arnan X, Blüthgen N (2015). Using ecophysiological traits to predict climatic and activity niches: lethal temperature and water loss in Mediterranean ants: Using physiology to predict niches. Glob Ecol. Biogeogr 24:1454–1464.

Arnan X, Blüthgen N, Molowny-Horas R, Retana J (2015). Thermal characterization of European ant communities along thermal gradients and its implications for community resilience to temperature variability. Front Ecol Evol 3:138.

Baudier KM, O'Donnell S (2016). Structure and thermal biology of subterranean army ant bivouacs in tropical montane forests. Insect Soc 63:467–476.

Bishop TR, Parr CL, Gibb H, Van Rensburg BJ, Braschler B, Chown SL, Foord SH, Lamy K, Munyai TC, Okey I, Tshivhandekano PG (2019). Thermoregulatory traits combine with range shifts to alter the future of montane ant assemblages. Glob Chang Biol 25:2162–2173.

Blomqvist MM, Olff H, Blaauw MB, Bongers T, Van Der Putten WH (2000). Interactions between above- and belowground biota: importance for small-scale vegetation mosaics in a grassland ecosystem. Oikos 90:582–598.

Bollazzi M, Kronenbitter J, Roces F (2008). Soil temperature, digging behaviour, and the adaptive value of nest depth in South American species of *Acromyrmex* leaf-cutting ants. Oecologia 158:165–175.

Braschler B, Duffy GA, Nortje E, Kritzinger-Klopper S, du Plessis D, Karenyi N, Leihy RI, Chown SL (2020). Realised rather than fundamental thermal niches predict site occupancy: Implications for climate change forecasting. J Anim Ecol 89:2863–2875.

Brian MV, Brian MV (1978). Production Ecology of Ants and Termites. (Cambridge University Press).

Cerda X, Retana J, Cros S (1998). Critical thermal limits in Mediterranean ant species: trade-off between mortality risk and foraging performance. Funct Ecol 12:45–55.

Chambers JM, Freeny A, Heiberger RM (1992). Analysis of variance; designed experiments. In *Statistical models in S* pp. 145–193.

Coppernoll-Houston D, Potter C (2018). Field measurements and satellite remote sensing of daily soil surface temperature variations in the lower Colorado Desert of California. Climate 6:94.

Dawkins R (2016). The Extended Phenotype: The Long Reach of the Gene. (Oxford University Press).

De Bruyn LAL, Conacher, AJ (1990). The role of termites and ants in soil modification-a review. Soil Res 28:55-93.

Deslippe RJ, Savolainen R (1995). Colony foundation and polygyny in the ant *Formica podzolica*. Behav Ecol Sociobiol 37:1–6.

Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR (2008). Impacts of climate warming on terrestrial ectotherms across latitude. Proc Natl Acad Sci USA 105:6668–6672.

Diamond SE, Sorger DM, Hulcr J, Pelini SL (2012). Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. Glob Chang Biol 18:448-456.

Fox J (2015). Applied Regression Analysis and Generalized Linear Models. (SAGE Publications).

Fowler HG (2008). Leaf-cuttings ants of the genera *Atta* and *Acromyrmex* of Paraguay (Hymenoptera: Formicidae). Mitt Mus Naturkunde Berl Dtsch Entomol Z 32:19–34.

Frouz J, Jílková V, Sorvari J (2016). Contribution of wood ants to nutrient cycling and ecosystem function. Wood ant ecology and conservation.

Ghalambor CK, Huey RB, Martin PR, Tewksbury JJ, Wang G (2006). Are mountain passes higher in the tropics? Janzen's hypothesis revisited. Integr Comp Biol 46:5–17.

Hansell M, Hansell MH (2005). Animal Architecture. (OUP Oxford).

Hillel D (1998). Environmental Soil Physics: Fundamentals, Applications, and Environmental Considerations. (Elsevier).

Hoffmann AA, Chown SL, Clusella-Trullas S (2013). Upper thermal limits in terrestrial ectotherms: how constrained are they? Funct Ecol 27:934–949.

Hölldobler B, Wilson EO (1990). The Ants. (Harvard University Press).

Huey RB, Stevenson RD (1979). Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. Am Zool 19:357–366.

Jílková V, Cajthaml T, Frouz J (2015). Respiration in wood ant (*Formica aquilonia*) nests as affected by altitudinal and seasonal changes in temperature. Soil Biol Biochem 86:50–57.

Jones CG, Lawton JH, Shachak M (1994). Organisms as ecosystem engineers. in Ecosystem management 130–147 (Springer).

Jouquet P, Dauber J, Lagerlöf J, Lavelle P, Lepage M (2006). Soil invertebrates as ecosystem engineers: Intended and accidental effects on soil and feedback loops. Appl Soil Ecol 32:153–164.

Kadochová Š, Frouz J, Roces F (2017). Sun basking in red wood ants *Formica polyctena* (Hymenoptera, Formicidae): Individual behaviour and temperature-dependent respiration rates. PLoS One 12:e0170570.

Kaspari M, Clay NA, Lucas J, Yanoviak SP, Kay A (2015). Thermal adaptation generates a diversity of thermal limits in a rainforest ant community. Glob Chang Biol 21:1092–1102.

Kearney M, Shine R, Porter WP (2009). The potential for behavioral thermoregulation to buffer 'cold-blooded' animals against climate warming. Proc Natl Acad Sci USA 106:3835–3840.

Khuong A, Gautrais J, Perna A, Sbaï C, Combe M, Kuntz P, Jost C, Theraulaz G (2016). Stigmergic construction and topochemical information shape ant nest architecture. Proc Natl Acad Sci USA 113:1303–1308.

Lapointe SL, Serrano MS, Jones PG (1998). Microgeographic and vertical distribution of *Acromynnex landolti* (Hymenoptera: Formicidae) nests in a neotropical savanna. Environ Entomol 27:636–641.

MacMahon JA, Mull JF, Crist TO (2000). Harvester ants (*Pogonomyrmex* spp.): Their community and ecosystem influences. Annu Rev Ecol Syst 31:265–291.

Mikheyev AS, Tschinkel, WR (2004). Nest architecture of the ant *Formica pallidefulva*: structure, costs and rules of excavation. Insect Soc 51:30–36.

Minter NJ, Franks NR, Brown KAR (2012). Morphogenesis of an extended phenotype: four-dimensional ant nest architecture. J R Soc Interface 9:586–595.

Penick CA, Tschinkel WR (2008). Thermoregulatory brood transport in the fire ant, *Solenopsis invicta*. Insect Soc 55:176–182.

Penick CA, Diamond SE, Sanders NJ, Dunn RR (2017). Beyond thermal limits: comprehensive metrics of performance identify key axes of thermal adaptation in ants. Funct Ecol 31:1091–1100.

Pinter-Wollman N (2015). Nest architecture shapes the collective behaviour of harvester ants. Biol Lett 11:20150695.

Porter SD (1988). Impact of temperature on colony growth and developmental rates of the ant, *Solenopsis invicta*. J Insect Physiol 34:1127–1133.

Roeder KA, Bujan J, Beurs KM, Weiser MD, Kaspari M (2021). Thermal traits predict the winners and losers under climate change: an example from North American ant communities. Ecosphere 12:e03645.

Rosengren R, Fortelius W, Lindström K, Luther A (1987). Phenology and causation of nest heating and thermoregulation in red wood ants of the *Formica rufa* group studied in coniferous forest habitats in southern Finland. Ann Zool Fennici 24:147–155.

Sala OE, Stuart Chapin FI, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R. Global biodiversity scenarios for the year 2100. Science 287:1770–1774.

Sankovitz MA, Breed MD (2019). Effects of *Formica podzolica* ant colonies on soil moisture, nitrogen, and plant communities near nests. Ecol Entomol 44:71-80.

Savolainen R, Vepsäläinen KA (1988). Competition hierarchy among boreal ants: Impact on resource partitioning and community structure. Oikos 51:135–155.

Schneider CA, Rasband WS, Eliceiri KW (2012). NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675.

Seeley T, Heinrich B (1981). Regulation of temperature in the nests of social insects.

Shik JZ, Arnan X, Oms CS, Cerdá X, Boulay R (2019). Evidence for locally adaptive metabolic rates among ant populations along an elevational gradient. J Anim Ecol 88:1240–1249.

Stockan JA, Robinson EJH (2016). Wood Ant Ecology and Conservation. (Cambridge University Press).

Talbot M (1934). Distribution of ant species in the Chicago region with reference to ecological factors and physiological toleration. Ecology 15:416–439.

Tschinkel WR (2003). Subterranean ant nests: trace fossils past and future? Palaeogeogr Palaeoclimatol Palaeoecol 192:321-333.

Tschinkel WR (2015). The architecture of subterranean ant nests: beauty and mystery underfoot. J Bioecon 17:271–291.

Wilson EO (1992). The effects of complex social life on evolution and biodiversity. Oikos 63:13–18.

Chapter III: Genomic signals of climate-associated selection in a widespread ant

Introduction

All species live within a geographic range, and the limits of this range may change over time. The extent of localities occupied is a species' realized ecological niche (Hargreaves et al. 2014). A central goal in evolutionary biology is to understand why some species undergo allopatric or parapatric speciation within their range while others continue to exchange genes (Slatkin 1987). Following this, we aim to understand why some species can adapt to changing conditions while others track their ecological niche (Ackerly 2003). Adaptation to new environments at range edges is necessary for niche expansion and adaptation to changing environments (Holt and Gomulkiewicz 1997). Theory predicts that range edges will harbor increased genetic isolation and differentiation, although range limit evolution is understudied; in particular, we know little about how gene flow and environmental factors shape species distributions (Sexton et al. 2009). Therefore, studying range margins can increase our understanding conditions limiting or encouraging species distribution growth.

The distribution of geographic range sizes spans from global (e.g. barn owl; Tomes et al. 2001) to small patches (e.g., desert fish; Gaston 1996). The ant genus *Formica*, for example, contains some species with ranges on a continental scale (e.g. *Formica podzolica*) and some that occupy ranges spanning only a few hundred kilometers (e.g. *F. francoeuri*) (Janicki et al. 2016; Guenard et al. 2017). Overall, the factors causing species to vary in range size remain largely unknown. Temporal trajectories, speciation patterns, and ecological determinants have significant theoretical

roles in shaping range size, although these ecological and evolutionary paradigms are difficult to test empirically (Gaston 1996; Hargreaves et al. 2014). Using rapidly improving technologies, we have only recently gained the ability to harness the power of genomic sequencing paired with population- and landscape-level analyses to investigate gene flow and adaptive potential at both the edges and centers of species ranges. Understanding how these dynamics play out across species ranges can inform us about the most influential factors shaping range size and range limits and allow us to predict range shifts under future climate projections (e.g. Jaffé et al. 2019).

Ants are among the most abundant insects, with high local diversity and globally widespread distribution (Hölldobler et al. 1990). Their ability to live in vastly different environments worldwide may be partly due to genomic adaptation to the local climate. Although genomic adaptation to climate has been identified in a wide variety of organisms (Hancock et al. 2011; Bay and Palumbi 2014; Lasky et al. 2015), it is unknown to what extent genomic variation in ants has been influenced by environmental factors.

In this study, I investigated the genetics of adaptation to climatic conditions in a widespread ant, *F. podzolica*. This ant's range extends throughout western North America, from Utah and New Mexico in the south to Alaska and the Yukon in the north (Janicki et al. 2016; Guenard et al. 2017). *Formica podzolica* live in various environments, making them an ideal species for investigating local climate adaptation, patterns of genetic distance, and how these dynamics may shape range limits. Integrating population genomics and environmental data across the range of this widespread species,

I investigated the questions: (1) *How do climatic conditions shape genomic variation across the range of* F. podzolica*? (2) How is this widespread ant species locally adapted to various climates? (3) Are patterns of genetic variation and adaptation different between range-edge and non-range-edge populations?*

Methods

Sample collection and DNA extraction

I started with 192 *Formica* samples from 49 locations across the species' range (latitudes 35.8196°N- 64.8886°N). These samples were deemed to be *F. podzolica* or a close relative based on their morphology and nest architecture. Members of the Purcell Lab collected these samples during 2016-2019 (Table S3.1). I extracted DNA from the samples using the Qiagen QIAamp 96 DNA QIAcube HT Kit and prepared libraries using the seqWell plexWell LP 384 next generation sequencing multiplexed library generation kit.

Whole-genome sequencing

Libraries for 192 *Formica* individuals were sequenced on an Illumina NovaSeq 6000 at Novogene. In addition, I obtained genomic sequences (FASTQ files) for 19 *F. podzolica* samples as prepared and used by Lagunas-Robles and colleagues (Lagunas-Robles et al. 2021). I used the software pipeline Stacks v2.1 (Catchen et al. 2013) to demultiplex, discard reads with low-quality scores, and trim adapters from the new sequences with the *process_radtags* function. I combined all the samples and merged overlapping paired-end

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reads of the combined dataset with the program PEAR v0.9.11 (Zhang et al. 2014),

aligned the reads to the *F. selysi* reference genome (Brelsford et al. 2020) using the Bwa-

mem2 v2.2.1 algorithm (Li 2013), and removed PCR duplicates with the program

Samtools v1.16 (Li et al. 2009). *Formica selysi* lives in Europe and shared a common

ancestor with *F. podzolica* ~18 Ma ago. There are few *Formica* reference genomes

available for use, so while *F. selysi* is the best reference genome option, the construction

of a reference genome for *F. glacialis,* the sister species of *F. podzolica*, is underway.

Increasing the phylogenetic distance between target species and the reference genome

can reduce the proportion of reads that successfully align, mapping quality, number of

SNPs generated, and depth at those SNPs (Bohling 2020). I called variants using the

function *mpileup* (Li 2011) and filtered genotypes for missing data (10% per locus, *--

max-missing 0.9*), minor allele count (*--mac 2*), and minimum depth (*--minDP 1*) using

the program package VCFtools v0.1.17 (Danecek et al. 2011). While including genotypes

with a depth of 1 means that I underestimated the number of heterozygous loci in each

sample, I used this filtering parameter to avoid losing SNPs that would be informative in

my analyses. I removed samples with more than 30% missing data and excluded

chromosome 3 because a large region of this chromosome harbors a supergene associated

with the social form of *Formica* ants and this region of the genome contained suppressed

recombination between sets of functional mutations for the past ~30 million years

(Purcell et al. 2021). Due to this differentiation, I plan to separately assess local

adaptation in chromosome 3 in a follow-up study. The original group of sequenced

individuals was made up of cryptic species, so to confirm the species identity of *F.*

podzolica I used PLINK v1.90b3.38 (*--distance square*) to create a distance matrix, which I then used in the program T-REX to construct a neighbor-joining tree including some individuals of known species (Purcell et al. 2007; Boc et al. 2012). I removed samples that were confirmed as species other than *F*. *podzolica* from the dataset. After filtering, I identified 1,700,356 single nucleotide polymorphisms (SNPs) in 152 *F*. *podzolica* individuals (Table S3.1).

Environmental data

For each sample, I obtained environmental data from publicly available databases. Specifically, I chose variables by plotting the two principal components that explained the most variation (68.59% combined) in the environmental data for my sample sites. Using this plot, I chose unconflated variables as those with large non-overlapping vectors. These variables, listed below with their abbreviations, included eight climate variables downloaded from the WorldClim data website (Hijmans et al. 2005). Additionally, I added land cover (U.S. Geological Survey 2002) and topsoil bulk density (Liu et al. 2014) to the environmental dataset.

  BIO1: Annual mean temperature

  BIO3: Isothermality (mean diurnal range / temperature annual range)

  BIO4: Temperature seasonality (standard deviation)

  BIO5: Max temperature of warmest month

  BIO10: Mean temperature of the warmest quarter

  BIO12: Annual precipitation

BIO15: Precipitation seasonality (coefficient of variation)

BIO17: Precipitation of the driest quarter

Population genetic analyses

To determine whether geographically distant populations show signs of historic isolation or recent expansion, I first inferred population structure using fastStructure (Raj et al. 2014) and used the *chooseK.py* function to choose model complexity (the most optimal K). Given K, fastStructure assigns individuals to clusters based on the posterior mean of admixture proportions. I calculated expected heterozygosity at variable sites (*--site-pi*, VCFtools; Danecek et al. 2011) for each population as the average nucleotide diversity per variable site. To investigate whether the genomic variation is more influenced by geography or environment across the range of *F. podzolica*, I calculated isolation-by-distance (IBD) and isolation-by-environment (IBE) between pairs of individuals. I calculated geographical great-circle distance (using the R package *pegas*; Paradis 2010) and genetic (Hamming) distances between pairs of individuals with the R package *poppr* (Kamvar et al. 2014), which counts the differences between genetic sequences. I combined elevation distance with Euclidean dissimilarities between all variables listed in the "environmental data" section above to calculate environmental distance. I scaled and centered environmental variables to equalize differences in magnitude (Lichstein 2007). I used Mantel tests and multiple regression of distance matrices (MRM) to examine the overall association between genetic and geographic or environmental distance. Specifically, I used separate Mantel tests to evaluate the correlation between genetic and

geographic or environmental distance. Both tests resulted in significantly positive correlations, so I used an MRM to perform regression analysis on the genetic distance to determine which predictor is the strongest force structuring genome-wide variation. To look at patterns at the range margins vs. the center of the range, I selected groups composed of 19-22 individuals from the northern-most latitudes, southern-most latitudes, and central-most latitudes with a minimum geographic distance of 0.09 km and an average distance of 121.99-326.62 km apart. I then calculated expected heterozygosity and used Mantel tests and MRMs as described above to assess IBD and IBE in each of these regional groups.

Identification of environmentally associated SNPs

To investigate the genomic basis of adaptation across the various heterogeneous environment of *F. podzolica*'s range, I identified genomic regions associated with the environmental variables mentioned above. To accomplish this, I used Samβada (Stucki et al. 2017), a software for landscape genomic analysis of large datasets that measures correlations between genotype and environmental variables while accounting for underlying population structure. I cross-validated the results from Samβada using latent factor mixed models (LFMMs), which also test for associations between genotypes and environments while accounting for background population structure. To prepare the genomic dataset, I used PLINK v1.90b3.38 to prune SNPs for linkage disequilibrium (LD) >0.4 r^2 (based on standards used in previous landscape genomic analysis using Samβada) (Vajana et al. 2018) with sliding windows of 200 SNPs and step size of 1 SNP

(option *–indep-pairwise 200 1 0.4*). After this pruning, 1,096,928 SNPs remained. For

each environmental variable, I used a bivariate model in Samβada with the environmental

variable of interest and a synthetic population structure variable including individual-

level admixture (as a second fixed effect) created by taking the first dimension from a

principal component analysis (PCA) on the SNP genotype data. Including population

structure reduces false positives in genotype-environment association tests (Storfer et al.

2018). I used log-likelihood ratio tests to identify markers significantly associated with

the selective pressure of interest. I corrected p-values for false discovery rate (FDR) using

the method of Benjamini & Hochberg (1995) to account for multiple tests. Finally, to set

a significance threshold, I used a permutation test in which I randomized the

environmental values associated with each sample over 100 runs of Samβada, calculated

individual p-values, corrected them for false discovery rate, and identified the minimum

p-value of each run. I assigned the fifth-smallest p-value (P=0.0013) of this ultimate set

as the significance threshold for my Samβada analyses. To ensure that the results from

Samβada were actual evidence of SNP/environment correlations, I further examined the

SNP that was most significantly associated with each environmental variable and six

SNPs that were highly (p<0.0001) associated with four environmental variables (BIO3,

BIO4, BIO12, and BIO17) using LFMMs. I used the least-squares estimation method

(*lfmm_ridge* function in the R package *lfmm*), and I adjusted for multiple tests using a

false discovery rate (FDR) correction (Caye et al. 2019). To assess whether the markers

putatively under selection showed a more positive relationship between genetic

differentiation and environmental dissimilarity, as expected for climate-associated loci, I

used Mantel tests and MRMs in the manner described above; however, I included genetic distance only from cross-validated markers putatively under selection. Finally, to identify any genes associated with outlier loci putatively under selection, I did a standard protein BLAST using the 800 nucleotides surrounding the SNP of interest and the National Library of Medicine's National Center for Biotechnology Information's (NCBI) *blastp* tool (Johnson et al. 2008).

Results

Population genetic analyses

fastStructure revealed two genetic clusters (K=2) across the species' full distribution range (Fig. 3.1). Both populations had relatively consistent expected heterozygosity (population 1: 0.1424; population 2: 0.1456). Similarly, ants at the northern, central, and southern portions of the range had relatively consistent expected heterozygosity (north: 0.1407, center: 0.1456, south: 0.1436). Overall, the pairwise genetic distance (Hamming distance) and geographic distance were highly correlated, suggesting a strong signal of isolation by distance (Fig. 3.2; Mantel's r = 0.5282, P = 0.0001). Genetic distance was also significantly correlated with environmental distance overall (Fig. 3.2; Mantel's r = 0.1131; P = 0.0006). In an MRM, both geographic and environmental distance were significant (R^2 = 0.5588; geography P = 0.001; environment P = 0.001). All regional groups showed patterns of isolation by distance, with the 10% northern-most individuals exhibiting lower IBD (Mantel's r = 0.2543, P = 0.0239) than the center (Mantel's r = 0.732, P = 0.0001) and southern (Mantel's r = 0.8905, P = 0.0001) groups (Fig. 3.4).

Only the central (Mantel's r = 0.6837, P = 0.0001) and southern groups (Mantel's r = 0.4264, P = 0.0001) showed patterns of isolation by environment through Mantel tests; the northern group exhibited no IBE (Mantel's r = 0.1405, P = 0.0867) (Fig. 3.4). MRMs revealed both geography and environmental distance as significant for both the central (R² = 0.3799; geography P = 0.003; environment P = 0.001) and southern group (R² = 0.5588; geography P = 0.001; environment P = 0.001). The southern group showed a lower genetic distance (at the intercept) than the northern and central groups (Fig. 3.4).
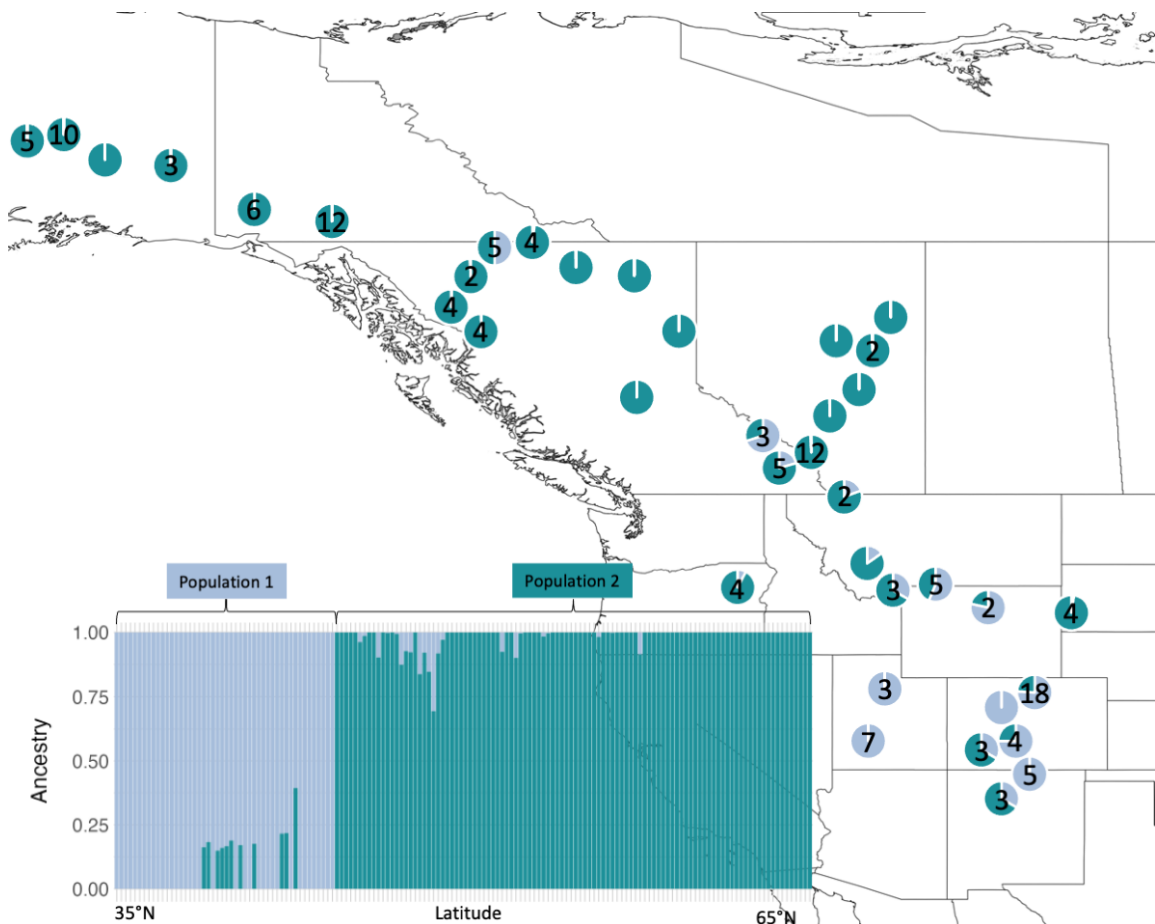

Figure 3.1. Map of the two populations as determined by fastStructure with admixture (ancestry) proportions shown in the bar plot.
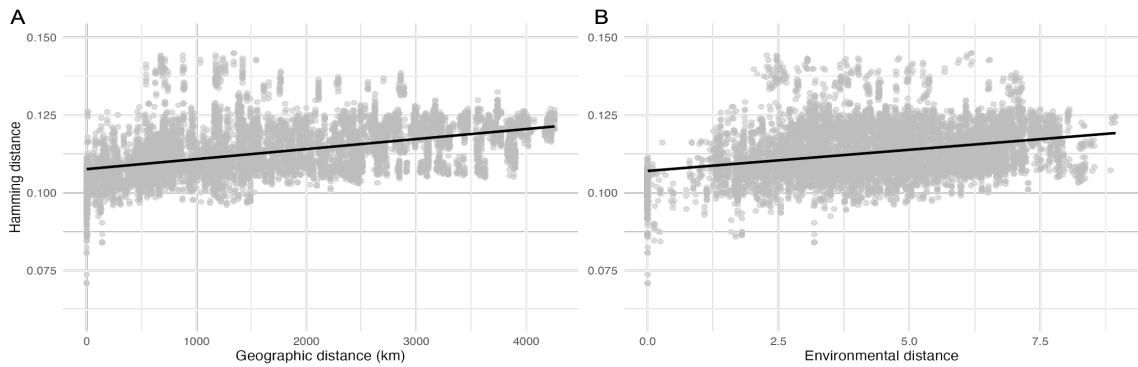
Figure 3.2. (a) Overall genetic isolation (Hamming distance) by geographic distance (km) and (b) environmental distance. Black lines show the best fit of the data points. The outliers with high genetic distance represent individuals from Oregon.

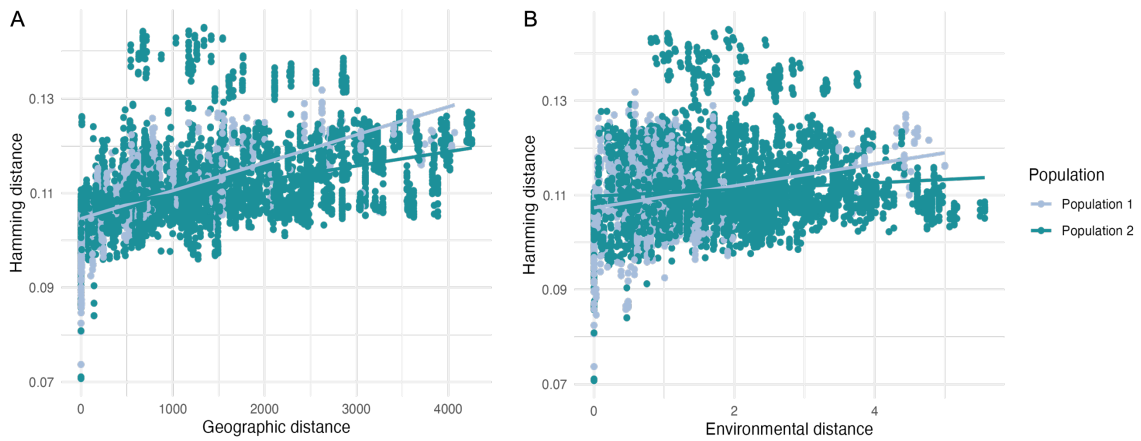Figure 3.3. (a) Genetic isolation (Hamming distance) by geographic distance (km) and (b) environmental distance of populations determined by fastStructure. Lines show the best fit for each population.
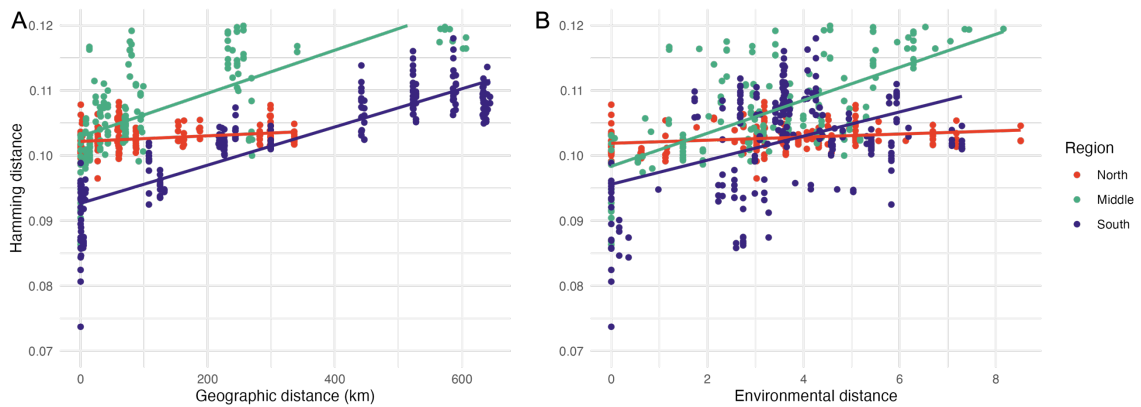

Figure 3.4. (a) Genetic isolation (Hamming distance) by geographic distance (km) and (b) environmental distance of the north, middle, and south regional groups. Lines show the best fit for each group.

Identification of environmentally associated SNPs

I found multiple SNPs significantly associated with climate variables BIO3, BIO4, BIO12, and BIO17 (Fig. S3.2) (FDR–corrected $P < 0.0013$). These markers were distributed across the genome. Upon cross-validation of the highest outlier for each environmental variable and the six SNPs that were highly ($p<0.0001$) associated with four environmental variables (BIO3, BIO4, BIO12, and BIO17), 20 SNP-environment correlations appear to potentially reflect local adaptation (Table S3.2). The SNP-environment correlations determined by Samβada that were not significant based on the LFMMs may reflect a false positive result or allele surfing during recent population expansions. Overall, outlier loci putatively under selection showed patterns of both IBD and IBE (MRM: $R^2 = 0.5247$; geography $P = 0.001*$; environment $P = 0.001*$) (Fig. 3.5). Outlier loci putatively under selection in the northern region showed no pattern of IBD (Mantel's $r = 0.0136$; $P = 0.4186$) or IBE (Mantel's $r = 0.0018$; $P = 0.4652$) (Fig. 3.6). Those in the central (MRM: $R^2 = 0.1612$; geography $P = 0.705$; environment $P = 0.001*$) and southern region showed a pattern of IBE (Mantel: geography $r = 0.0815$; $P = 0.13$; environment $r = 0.4288$ $P = 0.0001*$) (Fig. 3.6). BLAST search results indicated that all outlier loci putatively under selection are located within uncharacterized proteins.
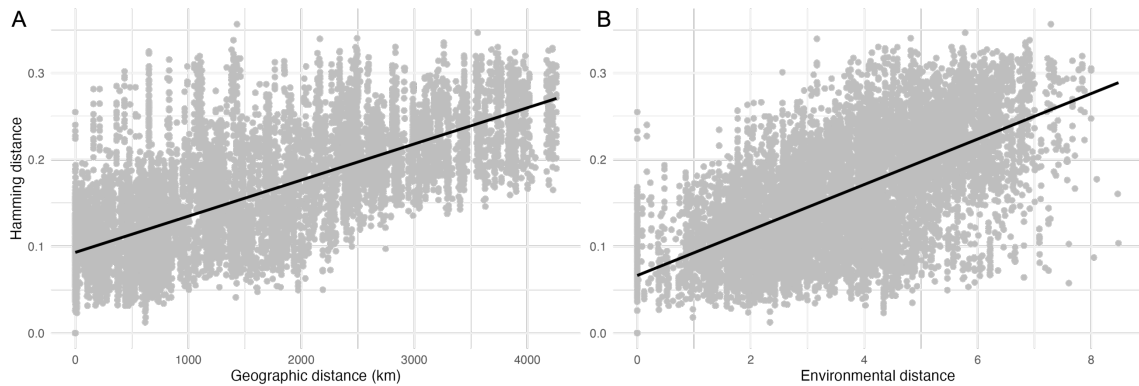
Figure 3.5. (a) Overall genetic isolation (Hamming distance) of outlier loci putatively under selection as a function of geographic distance (km) and (b) environmental distance. Black lines show the best fit of the data points.
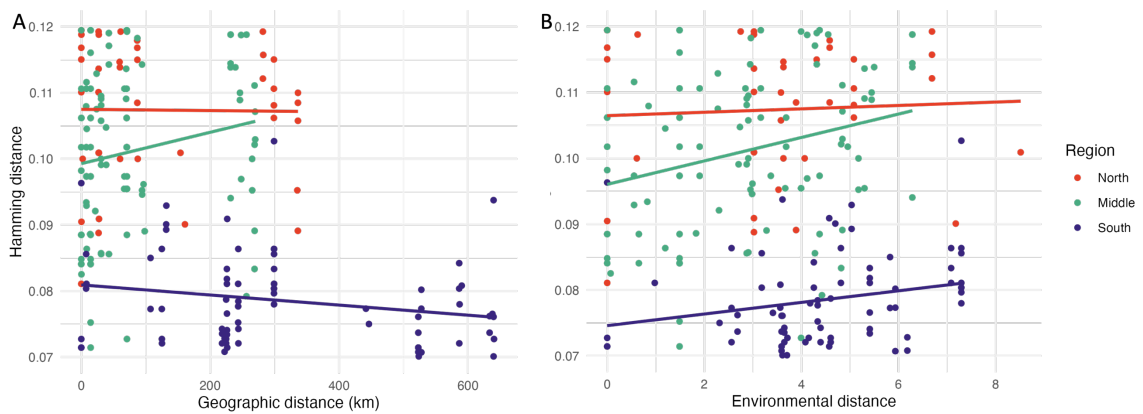
Figure 3.6. (a) Genetic isolation (Hamming distance) by geographic distance (km) and (b) environmental distance of outlier loci putatively under selection in the north, middle, and south regional groups. Lines show the best fit for each group.

Discussion

Here I describe genomic variation based on over a million genetic markers across the full range of a widespread ant species. Overall, there were two distinct genetic clusters across the range. While expected heterozygosity was consistent across the species' range, there was evidence of range wide IBD and IBE. Individuals in the central and southern regions of the range exhibited a pattern of genetic isolation by environment, whereas individuals

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in all regions exhibited genetic isolation by geographic distance. Finally, I identified 20 loci associated with isothermality, temperature seasonality, annual mean precipitation, and precipitation of the driest quarter.

fastStructure revealed two genetic clusters that explain the most structure in the data (Fig. 3.1); while the K=3 model revealed some additional substructure, it is not sufficient to suggest a third discrete population (Figure S1). This finding is consistent with range-wide isolation by distance (Eller 1999). Both IBD and IBE explained range-wide relatedness patterns. However, the dataset contains autocorrelation because the species range has a more extensive latitudinal distribution than longitudinal distribution, which causes the spatial pattern of IBD to align with the major north-south axis along which many climate factors vary. A more granular look at the northern, central, and southern regions (although these regions also contained autocorrelation at smaller scales) revealed that the individuals living at the northernmost extreme and center of the range showed higher within-site genetic distance (at the intercept) but similar expected heterozygosity to the individuals at the southernmost extreme. Further, IBE was present in the central and southern groups and absent in the north. The lower pairwise genetic distance within southern localities suggests that inbreeding or lower standing genetic variation within habitat islands may be present at the southern range limit, in the sky islands (a series of high mountains separated by lowland valleys) of the southwestern United States. High elevation climates make sky islands a specialized habitat, rendering them fragmented compared to more continuous habitat at lower elevations. Past climate change on these sky islands has been shown to increase genetic divergence in pine

butterflies due to limited dispersal ability and drive novelty in a core developmental gene network and its phenotype in the ant *Monomorium emersoni* (Favé et al. 2015; Halbritter et al. 2019). These results are consistent with a northwestward expansion of *F. podzolica* after glaciers receded in the Pleistocene (as occurred in montane spur-throated grasshoppers; Knowles 2000); as the species' distribution expanded and the geographic distance between subpopulations increased, the genetic distance between pairs of samples likely also increased (Quirk et al. 2022). The presence of IBD and absence of IBE at the northern end of the range of *F. podzolica* suggests that dispersal distance (rather than environment) is the strongest force structuring genomic variation between individuals and could be partially a result of continuous and homogeneous forest habitat. In contrast, the southern range edge is made up of isolated habitat patches wherein alpine forest only exists on mountaintops in the Sky Islands of New Mexico, constricting dispersal and potentially leading to inbreeding. A similar pattern of IBE has been found in white-breasted nuthatches in the Sky Islands (Manthey and Moyle 2015). However, the high genetic distance (at the intercept) in the north and relatively low genetic distance in the south are somewhat counterintuitive; it would be expected that small groups of isolated individuals within a fragmented habitat have a higher genetic distance across sites. Likewise, continuous habitat (such as in the north) would be expected to lead to high gene flow and, therefore, low genetic distance. Both local diversity (Hamming genetic distance between individuals in the same population) and regional genetic differentiation (genetic distance between individuals in different populations) are lower at the southern range limit (local: 0.1023; regional: 0.1014) than in the range center (local: 0.1074;

regional: 0.1159). Just regional differentiation (and not local diversity) is lower at the

northern range limit (local: 0.1127; regional: 0.1155) compared to the range center. The

difference between local patterns at the northern and southern range limit could reflect

differences in habitat connectivity (although this is not the only possible explanation;

sampling at a greater resolution on the population scale would reveal patterns of allele

frequency and Fst that would illuminate the demographic history of this species).

Several processes can contribute to the correlation between genetic differentiation

and environment: (a) local adaptation and selection against maladapted immigrants,

assuming local selection is relatively strong (Aeschbacher and Bürger 2014; Aeschbacher

et al. 2017); (b) biased dispersal of certain genotypes to a preferred habitat; and (c) IBD,

which I calculated separately from IBE in my analysis (Wang and Bradburd 2014). My

finding of range-wide isolation by distance is unsurprising because environmental

adaptation likely affects only a fraction of the genome (Bay et al. 2018). Biased dispersal

does not explain my results well because, although queens can choose specific

microhabitats for nest founding, the spatial scale of my sampling (distance between

neighboring sites: ~300 km +/- 100 km) is much larger than the dispersal distance of ant

queens (0.6 km +/- 0.2 km; Vitikainen et al. 2015). The dispersal distance of *F. podzolica*

queens in nature is unknown, but that of congeneric *F. exsecta* is estimated to be

approximately 600 m (Vitikainen et al. 2015).

Local adaptation, therefore, remains a reasonable explanation for at least some of

the genetic divergence observed across the range of *F. podzolica*. I found multiple

genotypes correlated with temperature, precipitation, and seasonality, possibly indicating

loci under selection. Precipitation is an environmental factor that has wide-ranging effects

on insects' physiological function, particularly in the context of desiccation stress (Beirne

1970; Addo-Bediako et al. 2001). Evidence suggests that the ant lifestyle is sensitive to

precipitation, and other species of ants are known to build their nests to control humidity

and protect the colony from precipitation (e.g. Bollazzi and Roces 2010a, 2010b). For

example, Texas leafcutter ants create environments suitable for fungal cultivars by

excavating garden chambers at soil depths with suitable humidity (Mueller et al. 2011).

Cuticular hydrocarbons, which serve as a waterproofing agent and communication signal,

have been found to depend on the precipitation in ants' habitats (Menzel et al. 2017). In

addition to precipitation, my results indicate that *F. podzolica* has loci adapted to

temperature and seasonality. Temperature has been found to affect *F. podzolica* nest

architecture and their trophic interactions with aphids (Nelson et al. 2019; Sankovitz and

Purcell 2021). Additionally, *Formica* ants are known to be sensitive to seasonal climate

fluctuations, exhibited through seasonal variation in nestmate recognition, colony odor

stability, and cuticular hydrocarbon profiles (Nielsen et al. 1999; Katzerke et al. 2006;

Martin et al. 2012).

While most of these loci had both or all alleles distributed throughout the species

range, one SNP (position 538106 on chromosome 14) had the alternative allele present

solely in the northernmost sites of the species range (Fig. 3.7 & 3.8). However, I did not

find a pattern of IBE among the outlier loci putatively under selection in the northern

region. This lack of IBE suggests that environmental adaptation is not actually at play in

SNP 14:538106 (false positive association), although the signal of IBE in this outlier may

just be overshadowed by the lack of signal from the others. Additionally, the environmental niche of the northern region is most explained by BIO4 (temperature seasonality) as revealed through PCA, not precipitation - as would be expected based on SNP 14:538106 (Fig. 3.9). An alternative explanation for the pattern observed in this locus is genetic drift, which theoretically occurs at a higher rate at range edges than the center of a range; therefore, drift via allele surfing may have been a contributing factor in the establishment of the putatively beneficial allele in the north (Klopfstein et al. 2006; Excoffier and Ray 2008; Sexton et al. 2009, 2014; Peischl et al. 2013).
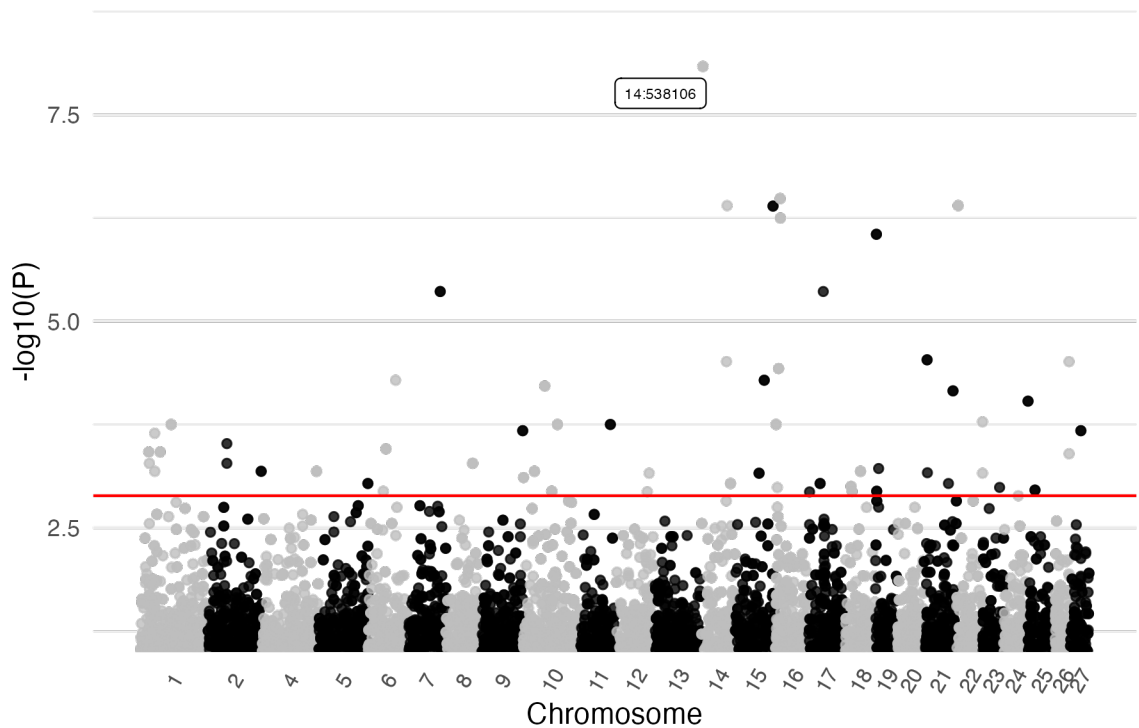

Figure 3.7. Samβada results for annual precipitation (BIO12). SNPs significantly correlated with annual precipitation are above the red line. The most significant outlier SNP is located on chromosome 14 at position 538106. Chromosome 3 was excluded from this analysis because a large region of this chromosome harbors a supergene associated with the social form of *Formica* ants.
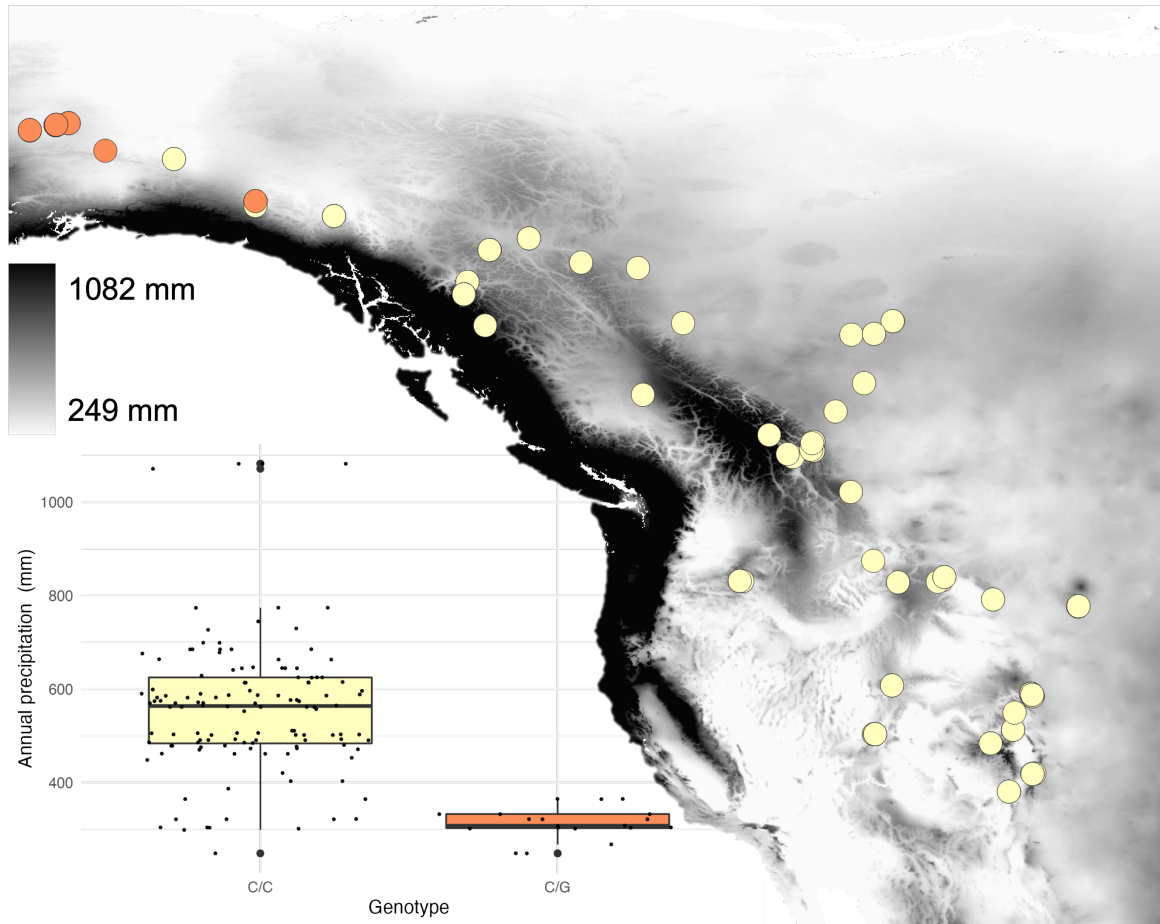
Figure 3.8. Map of annual precipitation (mm) with SNP alleles at position 538106 on chromosome 14. The boxplot shows genotype frequency with annual precipitation (mm). Outlier points are in bold on the boxplot. This SNP was the most significantly associated with annual precipitation from the Samβada analysis. The absence of G/G genotypes is likely a result of either genotyping error or the true absence of this genotype in this dataset; this locus has a higher-than-average mean read depth across all individuals (depth of 8.3355 compared to an average of 4.5940).

Figure 3.9. PCA plot showing vector weightings of the uncorrelated bioclimatic variables in relation to samples, which are colored by regional group.

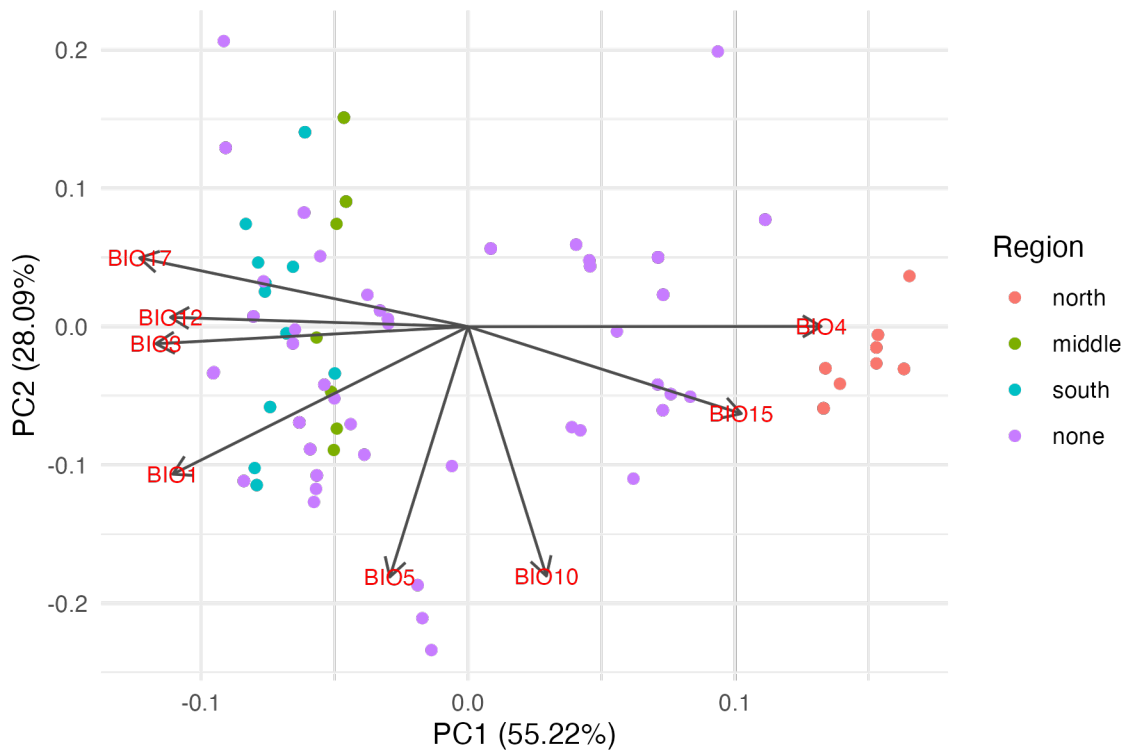Overall, my findings illuminate the range-wide and region-specific drivers of genetic distance in *F. podzolica* and suggest that temperature, precipitation, and seasonality are significant factors in habitat suitability for this species. Moreover, my work highlights previously unknown patterns of genetic isolation by distance and environment and possible local adaptation in these ants. Moving forward, these patterns of environmentally mediated evolution could inform predictions of *F. podzolica*'s response to climate change. This investigation could be achieved by measuring "genomic vulnerability", the mismatch between genotype and future environment, using associations across current gradients as a baseline. My results demonstrate how using both genomic and environmental data can offer a deeper understanding of how the

environment shapes genetic distance and adaptation, particularly at the range edges, of an

ant species with a broad latitudinal range.

References

Ackerly DD (2003). Community assembly, niche conservatism, and adaptive evolution in changing environments. Int J Plant Sci 164:S165–S184.

Addo-Bediako A, Chown SL, Gaston KJ (2001). Revisiting water loss in insects: a large scale view. J Insect Physiol 47:1377–1388.

Aeschbacher S, Bürger R (2014). The effect of linkage on establishment and survival of locally beneficial mutations. Genetics 197:317–336.

Aeschbacher S, Selby JP, Willis JH, Coop G (2017). Population-genomic inference of the strength and timing of selection against gene flow. P Nat Acad Sci USA 114:7061–7066.

Bay RA, Harrigan RJ, Underwood VL, Gibbs HL, Smith TB, Ruegg K (2018). Genomic signals of selection predict climate-driven population declines in a migratory bird. Science 359:83–86.

Bay RA, Palumbi SR (2014). Multilocus adaptation associated with heat resistance in reef-building corals. Curr Biol 24:2952–2956.

Beirne BP (1970). Effects of precipitation on crop insects. Can Entomol 102:1360–1373.

Benjamini Y, Hochberg Y (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Stat Soc 57:289–300.

Bohling J (2020). Evaluating the effect of reference genome divergence on the analysis of empirical RADseq datasets. Ecol Evol 10:7585-601.

Bollazzi M, Roces F (2010a). Leaf-cutting ant workers (*Acromyrmex heyeri*) trade off nest thermoregulation for humidity control. J Ethol 28:399–403.

Bollazzi M, Roces F (2010b). Control of nest water losses through building behavior in leaf-cutting ants (*Acromyrmex heyeri*). Insect Soc 57:267–273.

Brelsford A, Purcell J, Avril A, Tran Van P, Zhang J, Brütsch T, Sundström L, Helanterä H, Chapuisat M (2020). An ancient and eroded social supergene is widespread across *Formica* ants. Curr Biol 30:304–311.

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013). Stacks: an analysis tool set for population genomics. Mol Ecol 22:3124–3140.

Caye K, Jumentier B, Lepeule J, François O (2019). LFMM 2: fast and accurate inference of gene-environment associations in genome-wide studies. Mol Biol Evol 36:852-60.

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, McVean G, Durbin R, 1000 Genomes Project Analysis Group (2011). The variant call format and VCFtools. Bioinformatics 27:2156–2158.

Eller E (1999). Population substructure and isolation by distance in three continental regions. Am J Phys Anthropol 108:147–159.

Excoffier L, Ray N (2008). Surfing during population expansions promotes genetic revolutions and structuration. Trends Ecol Evol 23:347–351.

Favé MJ, Johnson RA, Cover S, Handschuh S, Metscher BD, Müller GB, Gopalan S, Abouheif E (2015). Past climate change on Sky Islands drives novelty in a core developmental gene network and its phenotype. BMC Evol Biol 15:1-21.

Gaston KJ (1996). Species-range-size distributions: patterns, mechanisms and implications. Trends Ecol Evol 11:197–201.

Guenard B, Weiser MD, Gomez K, Narula N, Economo EP (2017). The Global Ant Biodiversity Informatics (GABI) database: synthesizing data on the geographic distribution of ant species (Hymenoptera: Formicidae). Myrmecol News 24:83-89.

Halbritter DA, Storer CG, Kawahara AY, Daniels JC (2019). Phylogeography and population genetics of pine butterflies: Sky islands increase genetic divergence. Ecol Evol 9:13389-401.

Hancock AM, Brachi B, Faure N, Horton MW, Jarymowycz LB, Sperone FG, Toomajian C, Roux F, Bergelson J (2011). Adaptation to climate across the *Arabidopsis thaliana* genome. Science 334:83–86.

Hargreaves AL, Samis KE, Eckert CG (2014). Are species' range limits simply niche limits writ large? A review of transplant experiments beyond the range. Am Nat 183:157–173.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005). Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25:1965–1978.

Hölldobler B, Wilson EO (1990). The Ants. Harvard University Press.

Holt RD, Gomulkiewicz R (1997). How does immigration influence local adaptation? A reexamination of a familiar paradigm. Am Nat 149:563–572.

Jaffé R, Veiga JC, Pope NS, Lanes ÉCM, Carvalho CS, Alves R, Andrade SCS, Arias MC, Bonatti V, Carvalho AT. de Castro MS, Contrera FAL, Francoy TM, Freitas BM, Giannini TC, Hrncir M, Martins CF, Oliveira G, Saraiva AM, … Imperatriz-Fonseca VL (2019). Landscape genomics to the rescue of a tropical bee threatened by habitat loss and climate change. Evol Appl 12:1164–1177.

Janicki J, Narula N, Ziegler M, Guénard B, Economo EP (2016). Visualizing and interacting with large-volume biodiversity data using client–server web-mapping applications: The design and implementation of antmaps.org. Ecol Inform 32:185–193.

Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL (2008). NCBI BLAST: a better web interface. Nucleic Acids Research 36:W5-9.

Kamvar ZN, Tabima JF, Grünwald NJ (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281.

Katzerke A, Neumann P, Pirk CWW, Bliss P, Moritz RFA (2006). Seasonal nestmate recognition in the ant *Formica exsecta*. Behav Ecol Sociobiol 61:143–150.

Klopfstein S, Currat M, Excoffier L (2006). The fate of mutations surfing on the wave of a range expansion. Mol Biol Evol 23:482–490.

Knowles LL (2000). Tests of Pleistocene speciation in montane grasshoppers (genus *Melanoplus*) from the sky islands of western North America. Evolution 54:1337-48.

Lagunas-Robles G, Purcell J, Brelsford A (2021). Linked supergenes underlie split sex ratio and social organization in an ant. Proc Natl Acad Sci USA 118: e2101427118.

Lasky JR, Upadhyaya HD, Ramu P, Deshpande S, Hash CT, Bonnette J, Juenger TE, Hyma K, Acharya C, Mitchell SE, Buckler ES, Brenton Z, Kresovich S, Morris GP (2015). Genome-environment associations in sorghum landraces predict adaptive traits. Sci Adv 1:e1400218.

Lichstein JW (2007). Multiple regression on distance matrices: a multivariate spatial analysis tool. Plant Ecol 188:117–131.

Li H (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics 27:2987–2993.

Li H (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. In arXiv [q-bio.GN].

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup (2009). The Sequence Alignment/Map format and SAMtools. Bioinformatic 25:2078–2079.

Liu S, Wei Y, Post WM, Cook RB, Schaefer K, Thornton MM. (2014). NACP MsTMIP: unified North American soil map. ORNL DAAC.

Manthey JD, Moyle RG (2015). Isolation by environment in White-breasted Nuthatches (*Sitta carolinensis*) of the Madrean Archipelago sky islands: A landscape genomics approach. Mol Ecol 24:3628-38.

Martin SJ, Shemilt S, Drijfhout FP (2012). Effect of time on colony odour stability in the ant *Formica exsecta*. Die Naturwissenschaften 99:327–331.

Menzel F, Blaimer BB, Schmitt T (2017). How do cuticular hydrocarbons evolve? Physiological constraints and climatic and biotic selection pressures act on a complex functional trait. P Roy Soc B-Biol Sci 284: 20161727.

Mueller UG, Mikheyev AS, Hong E, Sen R, Warren DL, Solomon SE, Ishak HD, Cooper M, Miller JL, Shaffer KA, Juenger TE (2011). Evolution of cold-tolerant fungal symbionts permits winter fungiculture by leafcutter ants at the northern frontier of a tropical ant–fungus symbiosis. P Natl Acad Sci 108:4053–4056.

Nelson AS, Pratt RT, Pratt JD, Smith RA, Symanski CT, Prenot C, Mooney KA (2019). Progressive sensitivity of trophic levels to warming underlies an elevational gradient in ant–aphid mutualism strength. Oikos 128:540–550.

Nielsen J, Boomsma JJ, Oldham NJ, Petersen HC, Morgan ED (1999). Colony-level and season-specific variation in cuticular hydrocarbon profiles of individual workers in the ant *Formica truncorum*. Insect Soc 46:58–65.

Paradis E (2010). pegas: an R package for population genetics with an integrated–modular approach. Bioinformatics 26:419-420.

Peischl S, Dupanloup I, Kirkpatrick M, Excoffier L (2013). On the accumulation of deleterious mutations during range expansions. Mol Ecol 22:5972–5982.

Purcell J, Lagunas-Robles G, Rabeling C, Borowiec ML, Brelsford A (2021). The maintenance of polymorphism in an ancient social supergene. Mol Ecol 30:6246–6258.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, de Bakker PIW, Daly MJ, Sham PC (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81:559–575.

Quirk BJ, Huss E, Leonard E, Licciardi J, Plummer MA, Caffee MW (2022). Late Pleistocene glacial chronologies and paleoclimate in the northern Rocky Mountains. Clim Past 18:293-312.

Raj A, Stephens M, Pritchard JK (2014). fastSTRUCTURE: variational inference of population structure in large SNP data sets. Genetics 197:573–589.

Sankovitz M, Purcell J (2021). Ant nest architecture is shaped by local adaptation and plastic response to temperature. Sci Rep 11:23053.

Sexton JP, Hangartner SB, Hoffmann AA (2014). Genetic isolation by environment or distance: which pattern of gene flow is most common? Evolution; Int J Org Evol 68:1–15.

Sexton JP, McIntyre PJ, Angert AL, Rice KJ (2009). Evolution and ecology of species range limits. Annu Rev Ecol Syst 40:415-436.

Slatkin M (1987). Gene flow and the geographic structure of natural populations. Science 236:787–792.

Storfer A, Patton A, Fraik AK (2018). Navigating the interface between landscape genetics and landscape genomics. Front Gen 9:68.

Stucki S, Orozco-terWengel P, Forester BR, Duruz S, Colli L, Masembe C, Negrini R, Landguth E, Jones MR, NEXTGEN Consortium, Bruford MW, Taberlet P, Joost S (2017). High performance computation of landscape genomic models including local indicators of spatial association. Mol Ecol Resour 17:1072–1089.

Toms MP, Crick HQP, Shawyer CR (2001). The status of breeding Barn Owls *Tyto albain* the United Kingdom 1995–97. Bird Study 48:23–37.

Vajana E, Barbato M, Colli L, Milanesi M, Rochat E, Fabrizi E, Mukasa C, Del Corvo M, Masembe C, Muwanika VB, Kabi F, Sonstegard TS, Huson HJ, Negrini R, NextGen Consortium, Joost S, Ajmone-Marsan P (2018). Combining landscape genomics and ecological modelling to investigate local adaptation of indigenous Ugandan cattle to East Coast Fever. Front Genet 9:385.

Vitikainen EIK, Haag-Liautard C, Sundström L (2015). Natal dispersal, mating patterns, and inbreeding in the ant *Formica exsecta*. Am Nat 186:716–727.

Wang IJ, Bradburd GS (2014). Isolation by environment. Mol Ecol 23:5649–5662.

Zhang J, Kobert K, Flouri T, Stamatakis A (2014). PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30:614–620.

CONCLUSION

This research contributes to our understanding of ants' roles as ecosystem engineers in different environments and how they can survive in dramatically different climates across vast species ranges. I built on earlier work focused on single environments (e.g. Sanders and van Veen 2011; De Almeida et al. 2020) and greatly expanded our understanding of ant soil interactions and genomics across different environments. Observations from studies carried out in singular habitats are often extrapolated to entire species based on minimal empirical evidence across the distribution. Even fewer studies have taken a holistic approach in considering the many factors affecting ant fitness and their ability to transform their surrounding soil ecosystems. This research delves into some aspects of intraspecific variation in a widespread ant genus, investigating factors as diverse as behavioral plasticity, genetics, and an extended phenotype.

In Chapter I, using a large-scale, two-species study, I show that *Formica* ant nests differentially affect soil chemistry across elevational gradients. Specifically, I observed an interaction between soil carbon and nitrogen composition and elevation in each mountain range. At lower elevations, nest soil had lower amounts of carbon and nitrogen than control soil, but at higher elevations, nest soil had higher amounts of carbon and nitrogen than control soil. The nest soil moisture did not show any elevational patterns in either mountain range. However, my sampling method may only breach the interior of ant nests in some environments. I argue that ants likely modulate soil properties differently across environmental gradients but testing this effect must account for

variable nest architecture and other climate and landscape differences across diverse habitats.

In Chapter II, using a laboratory experiment, I show that the nest architecture of the ant *F. podzolica* is shaped by local adaptation and plastic response to temperature. Specifically, workers experiencing a high surface air temperature excavated deeper nests than those experiencing a cooler temperature. Further, I found evidence of local adaptation to temperature, with a significant interaction effect of natal elevation and temperature treatment on nest size and complexity. Specifically, workers from high elevation sites built larger nests with more tunnels when placed in the cool surface temperature treatment, and workers from low elevation sites exhibited the opposite pattern. I suggest that the plasticity of this 'extended phenotype' likely contributes to the widespread success of ants.

In Chapter III, in a landscape genomic study, I show that *F. podzolica* has two genetic clusters across the species distribution. Both geographic and environmental distance explained range-wide relatedness patterns, aligning with my findings of consistent expected heterozygosity across the range. Sites near the southern edge of the range showed lower pairwise genetic distance (at the intercept), possibly due to inbreeding and lower genetic distance before the distribution hypothetically expanded northward. Finally, I observed genomic signatures of adaptation to temperature, precipitation, and seasonality, with one locus exhibiting a precipitation-associated alternative allele only at the northern edge of the range. However, this may be a false positive association because outlier loci putatively under selection did not follow a

pattern of genetic isolation by environmental distance in the northern region. My findings

illuminate the range-wide and region-specific patterns of genetic distance in *F. podzolica,*

emphasize the role of the environment in shaping these patterns, and suggest that

temperature, precipitation, and seasonality are factors in habitat suitability for this

species.

Overall, the variation in phenotype, genotype, and environmental interactions in

Formica, like many other eusocial insects, is highly complex. The importance of ants in

soil ecosystems often centers around their functionality as ecosystem engineers (Kovář et

al. 2013; Meyer et al. 2013; Subedi 2016). While there is much evidence for the chemical

and physical soil alterations associated with subterranean-nesting ants that support

diverse microbiota, macroinvertebrates, and plants, my research in Chapter I shows that

these processes are highly dependent on environmental context. I designed my studies in

all three chapters around elevational gradients to take advantage of the climatic factors

that vary consistently and predictably with elevation: temperature and precipitation. As

this dissertation highlights, these variables shape ant behavior and play a significant role

in habitat suitability. However, as Chapter II exemplifies, ants are also uniquely

positioned to adjust to local temporal climate variation. Their ability to shape their nests

in direct response to surface air temperature highlights the significant advantage of this

extended phenotype, just one aspect of colony-based life that has led to their undoubted

global proliferation. Finally, Chapter III adds a larger evolutionary perspective,

suggesting that genetic changes are critical aspects of range expansion and species

adaptation to changing climates beyond the colony lifespan.

This research lays the groundwork for future studies on intraspecific variation in ant species with large distributions. A natural extension of Chapter I would entail disentangling the food web of the ant communities across the Sierra Nevada and San Jacinto mountains by examining C and N stable isotope ratios in the ants and associated insects, plants, and soil (e.g. Blüthgen et al. 2003). This stable isotope analysis would confirm the initial source of C and N and whether the observed differences in nest soil are a direct product of the ants. In a different future study, I would investigate how soil C and N composition influences ants, perhaps using a similar experimental setup as Chapter II. Instead of applying a temperature treatment, I would test how nest architecture possibly changes with soil containing different levels of C and N. Additionally, the design of Chapter I cannot distinguish between the effect of ants modifying the soil (habitat transformation) and non-randomly choosing where to build their nests (habitat choice). One way to test this would be to constrain newly mated queens within patches of soil of known C and N levels, enclosed within a fence barrier along an elevation gradient, and measure the same soil properties after the colonies have established nests. A follow-up study for Chapter II would entail using my custom nest boxes and temperature chambers to test the effects of soil type and the presence of vegetation on nest structure. Now that we know that ants can change their nest architecture in response to surface air temperature, it would be interesting to see what other abiotic and biotic factors impact their excavation behavior. Another variable to investigate would be social structure (i.e. whether the colony is monogyne or polygyne); colony and worker size are often associated with queen number, and these factors have the potential to influence nest

architecture. It would be interesting to elucidate whether social form impacts the nest

plasticity or level of local adaptation. Finally, future work building on Chapter III would

center around modeling future ant species distributions following the identification of

signatures of local adaptation to climate variables combined with future climate

projections (e.g. Bay et al. 2018).

The research in this dissertation is some of the first to consider the context of ant

ecosystem engineering and extended phenotypic plasticity in the context of

environmental variation. Additionally, very few landscape genomic studies have focused

on ants. Overall, there is much need for future research in these areas, and this

dissertation has illuminated some areas that could use attention and recommendations for

future researchers. My soil samples from Chapter I contained significant variability,

demonstrating the difficulty of taking representative soil samples inside ant nests. Despite

my efforts to homogenize the samples, analyzing mere grams of soil from an entire nest

probably does not capture a representative nest profile. I recommend that future

researchers studying ant-soil interactions sample soil from multiple locations within an

ant nest, both parallel and perpendicular to the soil surface. Moving forward, I plan to use

my data from Chapter III to continue to investigate the landscape genomics of *F.*

podzolica. The genomic signatures of environmentally mediated evolution I found could

inform predictions of *F. podzolica*'s response to climate change. This knowledge could

guide conservation actions, such as preserving mountain forests in suitable climates. I

plan to use my current data to predict which populations will be most vulnerable under

future climate scenarios. Ultimately, it is clear from this dissertation that intraspecies

genomic, phenotypic, and behavioral variation in ants is more dependent on

environmental factors than previously understood. I hope this work sheds light on this

complexity and encourages other social insect biologists to challenge the notion that

ecological, behavioral, and genomic studies carried out in a single environment are

sufficient for describing a species.

References

Bay RA, Harrigan RJ, Underwood VL, Gibbs HL, Smith TB, Ruegg K (2018). Genomic signals of selection predict climate-driven population declines in a migratory bird. Science 359:83–86.

Blüthgen N, Gebauer G, Fiedler K (2003). Disentangling a rainforest food web using stable isotopes: Dietary diversity in a species-rich ant community. Oecologia 137:426–35.

De Almeida T, Blight O, Mesléard F, Bulot A, Provost E, Dutoit T (2020). Harvester ants as ecological engineers for Mediterranean grassland restoration: Impacts on soil and vegetation. Biol Conserv 245:108547.

Kovář P, Vojtíšek P, Zentsová I (2013). Ants as ecosystem engineers in natural restoration of human made habitats. J Land Ecol 6:18-31.

Meyer ST, Neubauer M, Sayer EJ, Leal IR, Tabarelli M, Wirth R (2013). Leaf-cutting ants as ecosystem engineers: Topsoil and litter perturbations around *Atta cephalotes* nests reduce nutrient availability. Ecol Entomol 38:497–504.

Sanders D, van Veen FF (2011). Ecosystem engineering and predation: The multi-trophic impact of two ant species. J Anim Ecol 80:569–76.

Subedi IP (2016). Ants: Ecosystem engineers. Kist Newsletter 1:9-10.