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A Cross-Species Comparison of Task Partitioning in Ants
Lacking Discrete Morphological Worker Subcastes

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

Doctor of Philosophy

in

Entomology

by

Mari A. West

March 2022

Dissertation Committee:
      Dr. Jessica Purcell, Chairperson
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ABSTRACT OF THE DISSERTATION

A Cross-Species Comparison of Task Partitioning in Ants
Lacking Discrete Morphological Worker Subcastes

by

Mari A. West

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

Task partitioning allows for coordination of behavior in animal societies, potentially enhancing task efficiency. Many task allocation studies focus on social insects with discrete morphological worker subcastes, such as those possessing major and minor workers with strongly differentiated body plans. Much less is known about task partitioning among size-variable workers lacking discrete morphological subcastes, like in *Formica* ants. Through a large-scale mark-recapture study and a controlled laboratory experiment, we investigated how worker size affects task fidelity and proficiency across *Formica* species with differing degrees of body size variation. Additionally, we carried out genomic analyses to identify any genetic underpinnings of size-based task partitioning in these species. In species with high levels of worker size variation, a worker's body size is strongly correlated with the tasks it performs. Specifically, large workers specialize in nest building or protein foraging, while small workers specialize in honeydew collection. This size-task correlation is weaker, but still present, in species with less size variation among workers. Interestingly, our laboratory experiments suggest

that, in *Formica* species with substantial intracolony size variation, large workers outperform small workers at both nest building and sugar-water collection. It is unclear whether small workers' relatively poor performance at a task they typically perform in nature is due to limitations of our experimental design, or if small workers make other important contributions to colony efficiency. Genomic analyses reveal that both worker size and task may be under genetic control, although this is variable across species. The two phenotypes are not always genetically linked, although they appear to share some genetic associations in the most size-variable species analyzed. Combined, these studies suggest that *Formica* ants utilize a size-based task partitioning strategy, but the reliance on, benefits of, and genetic underpinnings of this strategy vary considerably across species. We expect social insects with varying degrees of morphological task specialization to differ in ontogeny, evolutionary history, and behavioral flexibility. Additional comparative studies will help us understand the potential costs and benefits of alternative strategies.

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Introduction

Background

Cooperation among organisms is widespread in nature and occurs both across and within species. In order for cooperation to be evolutionarily favorable, the benefits must ultimately outweigh the costs and lead to increased fitness (direct or indirect) for participants (Nowak 2006). In most cross-species mutualisms, there is an exchange of goods or services between organisms that leads to mutual benefit (Leigh Jr. 2010). For example, pollinators enable plant reproduction by transporting pollen among flowers in exchange for the nectar and pollen resources essential for their survival (e.g., Knauer & Schiestl 2015, Vandelook et al. 2019). Within species, cooperation among individuals may lead to either direct or indirect fitness benefits. For example, cooperative hunting allows groups to capture more and larger prey than any individual would be able to

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capture on its own, which can directly increase the survival and reproductive opportunity of group members (e.g., Lührs et al. 2013, Herbert-Read et al. 2016, Hubel et al. 2016, Grinsted et al. 2020). Similarly, individuals may gain indirect fitness benefits through participating in cooperative behaviors, like care and defense of others' young, if these behaviors increase the survival of their kin, which are more likely to propagate shared genetic material than non-kin (e.g., Eberle & Keppeler 2006, Uematsu et al. 2010, Konrad et al. 2019).

One form of intraspecific cooperation is division of labor, in which individuals or groups of individuals perform distinct tasks in parallel (e.g., Tizo-Pedroso & Del-Claro 2011, Mateus et al. 2019, Francioli et al. 2020), theoretically increasing colony efficiency (Jeanne 1986). In animal societies, division of labor takes two common forms - reproductive and non-reproductive. In reproductive division of labor, some individuals monopolize reproduction while others assist them in raising their offspring (e.g., Jarvis 1981; Walters et al. 1988; Terborgh & Goldizen 1985; van Kesteren et al. 2013). Among closely related individuals, reproductive division of labor theoretically promotes indirect fitness of helpers through increased success of their kin (Hamilton 1964), while unrelated helpers may enjoy direct benefits associated with a larger group size, increased opportunities for reproduction, and eventual inheritance of a shared nest (Clutton-Brock 2002). In non-reproductive division of labor, different individuals carry out distinct non-reproductive tasks, such as nest maintenance or foraging, that each contribute to their society's overall success. Non-reproductive division of labor may allow societies to

match individuals to tasks they are well suited for (e.g., Spaethe & Weidendmuller 2002, Arnan et al. 2011), thereby improving efficiency.

Division of labor is most well studied among the eusocial insects and is often identified as a key contributor to their widespread success. All eusocial insects live in groups and share three key traits: 1) cooperative brood care, 2) overlapping generations, and 3) reproductive division of labor (Wilson & Holldobler 2005). One or more fertile females ('queens') monopolize reproduction, while their daughters ('workers') carry out all non-reproductive tasks for the colony, including brood-rearing, resource collection, and defense (Keller & Nonacs 1993). Among workers, a non-reproductive division of labor also often occurs, wherein different groups of workers specialize in different tasks and perform them simultaneously, similar to workers in an assembly line. Task specialization theoretically allows individuals to gain the skills required to perform tasks proficiently and minimizes task-switching costs (Chittka & Muller 2009). However, the basis of task specialization is often complex, and studies that have investigated whether task specialization ultimately increases efficiency have led to mixed results.

Classically, workers are thought to specialize in tasks based on age (temporal polyethism) or morphology (morphological polyethism) (reviewed in Beshers & Fewell 2001). In temporal polyethism, young workers typically carry out safe, within-nest tasks while older workers perform risky, external tasks, with increased exposure to predators and pathogens (e.g., Nascimento et al. 2005, Torres et al. 2013). For example, honeybee workers begin their lives cleaning brood cells and rearing young and will gradually transition to nest guarding and foraging as they age (Free 1964; Johnson 2008). In

morphological polyethism, workers specialize in tasks based on body size and/or shape. For example, many ant species possess morphologically distinct, 'soldier' subcastes, which are characterized by disproportionately large heads and specialize in colony defense, while non-soldier workers perform all other tasks (e.g., Busher et al. 1985, Braendle et al. 2003, Powell 2008). Although less well studied, simple size variation among workers - without distinct morphological subcastes - can also be the basis of non-reproductive division of labor (e.g., bumblebees: Richards 1946, Cumber 1949, Brian 1952, Jandt & Dornhaus 2009; sweat bees: Breed et al. 1978; wasps: Spradbery 1972, O'Donnell & Jeanne 1995).

Many other important mechanisms contribute to task specialization and are often overlooked. For example, physiology plays a role in the development (e.g., Miura 2005) and glandular morphology of different morphological subcastes (e.g., Law et al. 1965), as well as behavioral maturation over time in temporal polyethism (e.g., Robinson 1987). Physiological differences can also be the basis of behavioral castes in same-age, same-size individuals (e.g., Huang et al. 1994; reviewed in Robinson 2009). Similarly, response thresholds to environmental cues vary among workers, leading to different behavioral responses from workers found in the same environment and predisposing workers to specialize in some tasks more than others (Theraulaz et al. 1998; Jeanson et al. 2008). Further, there is a molecular basis to task partitioning in many social insects. Differential gene expression can alter developmental pathways, producing workers of variable sizes or morphologies (Alvarado et al. 2015). Differing genetic lineages in polygyne (multi-queen) and polyandrous societies disproportionately contribute to workers of different

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morphological subcastes (Hughes et al. 2003), sizes (Schwander et al. 2005), task preferences (Julian & Fewell 2004), and response thresholds (Calderone & Page 1991; Page et al. 1998), suggesting that genetic diversity plays a role in task specialization.

In short, many factors combine to determine what task(s) an individual will perform. In honeybees (*Apis mellifera*), for example, physiological changes in hormone titers throughout individuals' lifetimes controls the rate of the transition from within-nest to external tasks as they age (Robinson 1987; Robinson et al. 1994). Additionally, individuals specialize in different tasks based on varying response thresholds (Pankiw & Page 2000), and this is correlated with different genetic lineages (Calderone & Page 1991; Page et al. 1998). Thus, age, physiology, response thresholds, and genetics all contribute to non-reproductive division of labor in honeybees. Similarly, in some social insects with morphological worker subcastes, physiology plays a major role in developmental patterning (Law et al. 1965; Miura 2005); in others, different genetic lineages disproportionally contribute to different morphological subcastes (Hughes et al. 2003). Many gaps remain, however, in our understanding of the mechanisms that contribute to task partitioning in social insects with workers with continuous, size variation and lacking discrete morphological worker subcastes. In this dissertation, I attempt to close this gap through examining the task partitioning strategy of an ant genus with continuously size variable workers and its evolutionary and genetic underpinnings.

Study System

Formica is a diverse ant genus that is widespread throughout the Earth's northern hemisphere (Francoeur 1973). Though most species inhabit forested habitats in northern latitudes, there are distinct taxonomic groups with long histories of independent evolution. For example, some *Formica* are entirely subterranean while others build large thatch mounds made out of pine needles, wood chips, grasses, or dirt atop their underground nest.

Formica is an excellent study system for investigating size-based non-reproductive division of labor for several reasons. First, all *Formica* that have been studied thus far possess continuously size-variable workers and lack discrete morphological worker subcastes (Tawdros et al. 2020). Second, the degree of size variability among workers differs across species, so that some species possess workers of dramatically different sizes (Fig. 0.1) while others possess workers that are less variable in size. This allows for comparisons between closely related species that may differ in their reliance on worker size variation for task partitioning. Third, *Formica* are socially polymorphic; in most species studied thus far, some colonies consist of a single queen and her offspring (monogyne) while others consist of multiple queens and their combined offspring (polygyne) (Keller 1993, DeHeer & Herbers 2004, Rosset & Chapuisat 2007, Zahnd et al. 2021). This allows us to investigate whether different maternal lineages contribute disproportionately to different worker specialists in polygyne colonies, as has been found in other systems. Additionally, this social polymorphism contributes to differences in worker size variability within single species. In *F. selysi*, for example,

workers from polygyne colonies are consistently smaller and less variable in size than those in monogyne colonies (Schwander et al. 2005). This allows for further comparisons within species, by assessing the role of size diversity in task partitioning in colonies that possess opposing social forms.

Figure 0.1: Two very differently sized *F. obscuriventris* workers collected from the same colony.

Research Objectives

Broadly, the fundamental goals of this dissertation were to characterize the task allocation strategy used by a continuously size-variable social insect and assess its potential evolutionary and genetic underpinnings. For each chapter, I compared several *Formica* species that differ in the degree of size variation among workers to:

I.   assess the degree of task fidelity among *Formica* and investigate associations between worker size and task,

II.   determine whether worker size influences task proficiency, and

III.   identify the role of genetics in controlling worker size and task performance.

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Chapter I: Task partitioning in ants lacking discrete morphological worker subcastes

Introduction

In animal societies, cooperation between individuals confers benefits such as increased survival, reproduction, access to shared resources, and success of kin (reviewed in Clutton-Brock 2002). Cooperative individuals may benefit from division of labor, enabling the performance of multiple tasks in parallel. Division of labor can be either reproductive or non-reproductive. In reproductive division of labor, some individuals forego reproduction and instead aid other group members in raising their offspring (e.g. Jarvis 1981; Walters et al. 1988). For example, in many species of cooperatively breeding birds, reproductively mature individuals act as "helpers," providing alloparental care prior to inheriting breeding status (Walters et al. 1988). In non-reproductive division of

labor, individuals often specialize in a single or a few similar tasks, such as resource collection or defense. In many cases, non-reproductive division of labor is accomplished without any central control directing the behavior of individuals. In such cases, task specialization is mediated by a combination of internal and environmental cues (Gordon 1996). Task specialization increases the functional efficiency of societies by enabling individuals to effectively learn skills associated with a small suite of tasks, transfer skills when performing similar tasks, and avoid costs associated with switching tasks (Oster & Wilson 1979; Chittka & Muller 2009).

In line with the idea that task specialization increases efficiency, the ecological success of social insects is often attributed to division of labor (Oster & Wilson 1979). Eusocial insects are incredibly successful. Ants and termites alone, though representing only 2% of insect species, comprise more than half of all insect biomass worldwide and approximately one-third of all animal biomass in tropical systems (Hölldobler & Wilson 1990; Wilson & Hölldobler 2005a). Eusocial insects live in groups of closely related individuals and are characterized by cooperative brood care, overlapping generations, and reproductive division of labor (Wilson & Hölldobler 2005b). All eusocial insects use some form of reproductive division of labor, in which one or more egg-laying queens focus primarily on reproduction, while workers carry out all other tasks within the colony, including nest maintenance, brood care, and foraging (Wilson 1971). These and other non-reproductive tasks are divided among workers in most insect societies. Natural variation among workers enables them to specialize in one or a few tasks effectively based upon their individual characteristics (Wilson 1968; Hasegawa 1997).

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One form of non-reproductive division of labor, morphological polyethism, relies on morphological variation among workers. In a broad sense, morphological polyethism is defined as task specialization on the basis of variation in the size and/or shape of workers (reviewed in Beshers & Fewell 2001; Robinson 1992). Within eusocial Hymenoptera, morphological polyethism has been identified in at least 15% of ants (Oster & Wilson 1979) and in some stingless bees (Grüter et al. 2012; Grüter et al. 2017). A kind of morphological polyethism is also present in most termites, but the fundamental differences in polyethism that arise from their hemimetabolous development, such as specialization based on developmental stage, are beyond the scope of this paper (Noirot & Pasteels 1987). Morphological polyethism has been studied primarily in ants with non-linear allometric scaling among workers, wherein the log-log allometric regression is broken into two or more linear associations with different slopes (Wilson 1953). In these species, workers that perform different tasks, not only differ in overall size, but also differ from each other in the relative proportions or scaling of their body plan. For example, workers of many *Pheidole* species are split into a major (or soldier) subcaste, which specialize in colony defense, and a minor subcaste, which performs brood care and foraging (Mertl & Traniello 2009). Throughout this manuscript, we refer to these species as those possessing 'discrete morphological worker subcastes.' However, many hymenopteran social insect species lack discrete morphological worker subcastes (Wilson 1953; Oster & Wilson 1979; Harvell 1994), but still exhibit substantial variation in body size. Much less is known about how these species partition tasks.

In some size-variable social insects lacking discrete morphological worker subcastes that have been investigated, an association between task specialization and worker size is noticeable. In some cases, close examination has revealed subtle changes in body shape associated with body size, as in some stingless bees (Grüter et al. 2012; Grüter et al. 2017) and some fire ants (Tschinkel et al. 2003; Tschinkel 2013). Allometric scaling relationships among workers of these species are primarily linear, meaning that the same allometric scaling relationships exist across all body sizes. Worker size variation is also often associated with differences in timing of task performance and task repertoire. Within bumble bees (Richards 1946; Brian 1952) and a few *Vespula* and *Polybia* wasp species (Spradbery 1972; O'Donnell & Jeanne 1995), for example, large workers tend to perform exterior tasks earlier or more frequently than small workers, but task specialization is weak overall (Jandt et al. 2009). As size variation among workers is likely present in most insect societies, it is possible that size-based task allocation systems are relatively common, even though they are relatively understudied. In order to understand how widespread size-based task partitioning is, it is important to understand how the degree of size variation among nestmate workers impacts this strategy.

The ant genus *Formica* is widespread throughout North America, Europe, and Asia (Bondroit 1918; Dlussky 1965; Bernard 1968; Francoeur 1973). Species of this genus lack discrete morphological worker subcastes and workers within colonies are continuously variable in size (e.g. Bernstein 1976; Billick 2002; Schwander et al. 2005; Fig. S1.4; Fig. S1.5). Comparison of body measurements in some species has revealed linear allometric (and some isometric) scaling relationships associated with body size

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(Tawdros et al. 2020). Previous studies suggest that *Formica* exhibits morphological polyethism, such that size variation among workers is associated with task allocation (Herbers 1979; Bernstein 1976; Batchelor et al. 2012; Parmentier et al. 2015; Véle & Modlinger 2019). Additionally, Billick and Carter (2007) found that lab colonies with natural worker size variation were better able to maintain colony biomass (i.e. worker survival) than those with only small or only large workers. However, little is known about task fidelity in *Formica*, which is important for understanding the strength of the association between morphology and behavior in this system. Across *Formica* colonies and species, the magnitude of variation in worker size can vary drastically, allowing us to ask how much size variation is needed to yield this task allocation strategy and whether task switching is more common in colonies with less size variance. Few studies have investigated task allocation strategies in closely related species that differ in the degree of size variation or polymorphism (but see Wilson 1978).

Formica provides an opportunity to investigate the factors contributing to task allocation in social insects with high levels of natural worker size variation and enables us to make direct comparisons between related species that differ in the degree of this size variation. The main goals of this study were to determine whether 1) individual *Formica* workers specialize in single tasks over short periods, 2) there is an association between worker size and task across diverse species in this genus, and 3) within-colony size variation impacts the strength of the size-task association. We conducted a multi-species mark-recapture study, investigating behavioral fidelity and worker size associated with individual task performance.

Materials and Methods

       In June - August of 2017, we conducted behavioral observations on and collected specimens from 51 *Formica* ant colonies. We observed colonies of ten species at ten localities distributed throughout southern Alberta, Canada (Table 1.1). It was not possible to collect data blindly, as our study involved focal ant workers in the field.

Table 1.1: A list of the localities in Alberta, Canada where we conducted observations and collected samples. The number of colonies observed per species is indicated in parentheses after each species name.

Locality	Latitude (°N)	Longitude (°W)	Number of Colonies	Species
Barrier Lake Day Use Area	51.03	-115.04	5	*F. obscuriventris* (3), *F. neoclara* (2)
Castle Provincial Park	49.38	-114.35	2	*F. aserva* (1), *F. rufa sp. #1* (1)
Exshaw	51.08	-115.12	3	*F. dakotensis* (2), *F. neoclara* (1)
Grassi Lakes Day Use Area	51.08	-115.4	2	*F. neoclara* (1), *F. rufa sp. #2* (1)
Nose Hill Park	51.11	-114.1	3	*F. glacialis* (1), *F. neoclara* (1), *F. obscuriventris* (1)
Peter Lougheed Provincial Park	50.69	-115.13	3	*F. neoclara* (1), *F. neorufibaris* (1), *F. podzolica* (1)
Sibbald Lake Provincial Campground	51.05	-114.86	2	*F. aserva* (1), *F. ulkei* (1)
Sylvan Lake Provincial Park	52.35	-114.08	15	*F. glacialis* (6), *F. podzolica* (4), *F. aserva* (3), *F. neoclara* (1), *F. ulkei* (1)
University of Calgary Biogeosciences Research Station	51.03	-115.03	14	*F. obscuriventris* (7), *F. podzolica* (4), *F. neoclara* (3)
Wasootch Creek Day Use Area	50.97	-115.09	2	*F. obscuriventris* (1), *F. podzolica* (1)

Mark-Recapture

We observed each colony over two days, for a total of four hours per colony. We split each day into four half-hour observation periods, spread throughout the day in order

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to capture each colony's active period and to minimize any temporal biases. Due to weather constraints, we observed three colonies for three or three and a half hours.

We focused on three tasks: honeydew collecting (HC), nest building (NB), and protein foraging (PF). Honeydew collectors tended to aphids or departed an aphid-infested plant with fully engorged abdomens. Nest builders carried nest materials (e.g. pine needles, wood chips, dirt, pebbles) on or adjacent to the nest mound or entrance. Protein foragers carried prey (a variety of insects and spiders) toward the nest entrance. We chose these three tasks because they consistently had a large proportion of the workforce allocated to them across colonies and observations did not require nest destruction. It was critical for the success of our mark-recapture study to maintain the integrity and normal activity of the observation nests. While one researcher observed and paint-marked nest builders and protein foragers at the nest, the other observed and paint-marked honeydew collectors at a nearby aphid-infested plant.

On the first day of observation, we paint-marked individuals observed doing one of the previously described tasks with a task-associated color. We randomized color-task associations for each colony through blindly choosing paints from a collection of 12 Testors® enamel paints. Using a toothpick, we painted a small dot on the gaster of each individual. If we observed a previously painted worker switching tasks, we added another dot of paint of a different color. We used manual counters to keep track of the number of individuals painted for each task during each observation period and noted any workers observed switching tasks.

On the second day of observation, which was usually the following day (N = 45 nests; 3 days later, N = 3 nests; 4 days later, N = 3 nests), we recaptured painted individuals observed doing the same or a different task into a 26.7 x 20.3 x 15.2 cm plastic bin, lined with fluon to prevent escape. We used manual counters to keep track of the number of workers recaptured doing the same task. We also noted any workers recaptured doing a different task. From the recapture bins, we haphazardly collected up to 15 of the recaptured workers performing the same task and all workers observed switching tasks for head-width measurements. Hereafter, we refer to these as 'consistent workers' and 'task-switchers,' respectively. We stored consistent workers (by task) and task-switchers separately in 100% ethanol. Additionally, we collected up to 15 unmarked nest builders, protein foragers, and honeydew collectors from each colony into 100% ethanol when feasible.

Head Measurements

We used a Leica S8AP0 microscope with a Leica DMC2900 camera attached and Leica imaging software to photograph the head of each specimen at 25x magnification. We then used the Leica imaging software to measure the head width of each specimen to one-thousandth of a millimeter, measuring the widest point across the eyes (Fig. S1.1). We chose to measure head width because it is a good proxy for overall body size in *Formica selysi* (Schwander et al. 2005) and other ants (e.g. Kaspari 1996; Fournier et al. 2008).

Species Identification

We used genetic sequencing (RADseq) to verify field identification of species collected for this study. Please see the supplementary material for a full description of relevant methods and a neighbor-joining tree showing relationships between sampled nests (Fig. S1.6). In total, we observed ten *Formica* species during our mark-recapture study (Table 1.1). We were unable to identify two species in the *F. rufa* group. These are denoted as '*F. rufa sp. #1*' and '*F. rufa sp. #2*' in Table 1.1.

For the purposes of this study, we separated the species into two groups: mound-building and subterranean species. The distinction between these two groups is based primarily on phylogeny (Fig. S1.6). However, it is important to note that the two groups generally differ in the degree of worker size variation and many aspects of their life histories. For example, the mound-building species included in this study are facultative social parasites, while the subterranean species are common hosts (e.g. Savolainen & Deslippe 2001). The use of the 'mound-building' and 'subterranean' descriptors throughout this manuscript refers to the external view of nests built by each species; mound-building species typically build thatch mounds with conifer needles and bark or dried grasses atop their underground nests (e.g. Scherba 1961), while subterranean species' nests are either entirely underground or include an aboveground dirt mound (e.g. Sankovitz et al. 2019).

Statistical Analyses

We performed all statistical analyses in R version 3.5.2 (R Core Team 2017). In order to compare recapture rates and task dynamics across species and task groups, we performed a series of Fisher's exact tests using the *fisher.test* function.

In the primary size-task association analyses, we included the species for which we had more than one colony and five or more workers per task (36 of 51 colonies, six of ten species). The 36 colonies included in the size-task association analyses are members of three mound-building species, with generally high worker size variance (*F. aserva*, *F. dakotensis,* and *F. obscuriventris*) and three subterranean species, with low worker size variance (*F. glacialis, F. neoclara*, and *F. podzolica*). We used the *lmer* function from the lme4 package, with the lmerTest modification (Bates et al. 2014; Kuznetsova et al. 2015) to build six linear mixed effects models (one for each species) in order to compare the head widths of individuals that performed different tasks. In these models, head width was the response variable, task was the fixed effect, and colony was the random effect. As a post hoc analysis, we performed Tukey's range tests in order to compare average head widths across all task categories for each species using the *multcomp* package (Hothorn et al. 2017). In addition to these primary analyses, we carried out analyses on the remaining four species but note that we had lower statistical power due to small sample sizes (Supplementary Methods; Fig. S1.3; Table S1.1).

To assess the impact of within-colony worker size variance on the strength of size-task associations, we carried out a phylogenetic independent contrast (PIC) for each task comparison (NB-HC, HC-PF, and PF-NB). We carried out the PICs using a total of

48 colonies for which we had sufficient genomic, behavioral, and morphological data. We excluded two colonies for which we lacked head width measurements for at least two workers per task from at least two of the three focal tasks and one colony for which we lacked genomic data. As such, we included a variable number of colonies in each PIC: 47 colonies when comparing honeydew collectors to nest builders, 46 colonies when comparing nest builders to protein foragers, and 45 colonies when comparing honeydew collectors to protein foragers. We calculated the effect size (Cohen's d) of each task comparison per colony using the *cohen.d* function from the effsize package (Torchiano & Torchiano 2018) and within-colony head-width variance and then log-transformed the data. We generated neighbor-joining trees based on genomic data from one worker per colony (see supplementary material for detailed genomic methods). We chose to carry out these analyses across colonies, since our conspecific samples were collected from different populations (Table 1.1), and we observed substantial intraspecific variation in some species (Fig. S1.2; Fig. S1.6). We used PLINK (Purcell et al. 2007) to calculate genetic distance matrices for the colonies associated with each PIC. We then generated neighbor-joining trees in the Newick format using the web-based platform T-REX (Boc et al. 2012). The trees were not dichotomous so we rerooted them using the *midpoint.root* function in the phytools package (Revell 2012). Phylogenetic comparative methods appear to be robust to tree misspecification, so it is unlikely that rerooting the trees significantly impacted the results of the PICs (Stone 2011). We then examined the correlation between within-colony size variance and effect size for each task comparison while correcting for phylogeny using the *pic* function from the ape package (Paradis &

Schliep 2019). Within-colony variance in head-width was the fixed effect and Cohen's d was the response variable.

Data Availability

The behavioral and morphological data generated and analyzed for this study are available on Dryad, https://doi.org/10.6086/D1D965. The genomic data generated and analyzed for this study are available on NCBI GenBank, https://www.ncbi.nlm.nih.gov/bioproject/SUB6698223.

Results

Mark-Recapture

During our mark-recapture study, we marked 8831 ant workers (HC, N=6074; NB, N=1689; PF, N=1068). On subsequent observation days, we recaptured 3,570 (40.4%) marked ants (HC, N=3276; NB, N=241; PF, N=53). We carried out several Fisher's exact tests to compare recapture and task fidelity rate across species and focal tasks. Across all species, the honeydew collectors were more likely to be recaptured than the nest builders ($p<0.0001$) and protein foragers ($p<0.0001$), and the protein foragers were less likely to be recaptured than the nest builders ($p<0.0001$). Of those recaptured, 3520 (98.6%) were consistent workers (HC, N=3260; NB, N=222; PF, N=38), and 50 (1.4%) were task-switchers (HC, N=16; NB, N=19; PF, N=15) (Fig. 1.1). Overall, recaptured workers from the subterranean species were more likely to switch tasks than those from the mound-building species ($p=0.008$). Of the 1792 recaptured subterranean workers, 34 (1.9%) switched tasks (Fig. 1.1B), while only 16 out of 1778 (0.9%)

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recaptured workers from the mound-building species switched tasks (Fig. 1.1A). Within the mound-building species, the honeydew collectors were more likely to maintain task fidelity than the nest builders (p=0.002) and protein foragers (p<0.0001), and the protein foragers were more likely to switch tasks than the nest builders (p<0.0001; Fig. 1.1A). Within the subterranean species, the honeydew collectors were also more likely to maintain task fidelity than the nest builders (p<0.0001) and protein foragers (p<0.0001), but the nest builders and protein foragers were equally likely to switch tasks (p=0.419; Fig. 1.1B). Additionally, two subterranean workers that collected honeydew on the first day of observation were observed performing a non-focal task on the second day of observation – one was removing a dead worker from the colony and the other was observed carrying an aphid.

We revisited two colonies about one month after initial markings. We observed approximately 15 painted honeydew collectors tending to aphids in both cases. This observation suggests that workers consistently perform this task for at least one month. We did not observe previously painted nest builders or protein foragers carrying out any focal tasks.
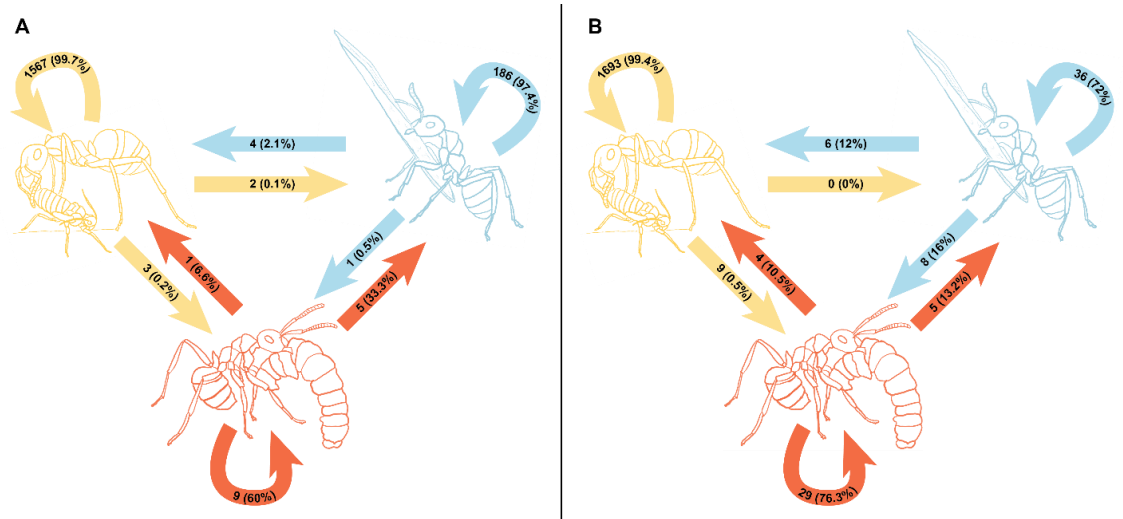
Figure 1.1: Diagram depicting the number of workers that were recaptured collecting honeydew (HC, yellow), nest building (NB, blue), and protein foraging (PF, orange) for mound-building (A) and subterranean (B) species. For each arrow, the blunt end indicates the task observed when workers were initially marked, and the point indicates the task observed when workers were recaptured. As such, curved arrows represent consistent workers and straight arrows between tasks represent task-switchers. Raw numbers are followed by the percentages of recaptured honeydew collectors, nest builders, or protein foragers that were observed performing the tasks indicated by the direction of the arrow.

Size and Task Partitioning

We find a consistent pattern when considering associations between worker size and task in the mound-building species (*F. obscuriventris, F. aserva*, and *F. dakotensis*). For these species, honeydew collectors are significantly smaller than both nest builders and protein foragers, which are not significantly different in size from each other (Fig. 1.2: A, $F_{2,189}$=144.6, p<0. 0001; B, $F_{2,67}$=30.53, p<0.0001; C, $F_{2,43}$=9.76, p=0.0003). These trends are consistent when considering workers within colonies of each mound-building species (Fig. S1.2A). We find slightly different patterns when considering the association between size and task in the subterranean species (*F. podzolica*, *F. glacialis*, and *F. neoclara*). For all three species, honeydew collectors are small, but the differences between nest builders and protein foragers varies (Fig. 1.2: D, $F_{2,196}$=4.26, p=0.015; E,

$F_{2,159}=5.18$, p=0. 007; F, $F_{2,155}=3.67$, p=0.028). Overall, there is a weaker relationship between worker size and task in the subterranean *Formica* included in our study. In contrast with the mound-building species, the relationship between worker size and task differs among subterranean colonies of the same species (Fig. S1.2B). The presence of intraspecific body size variation across colonies within the subterranean species likely contributes to weaker associations between size and task overall (Fig. S1.6). Size-task associations for the four additional species are provided in the supplementary material (Fig. S1.3).
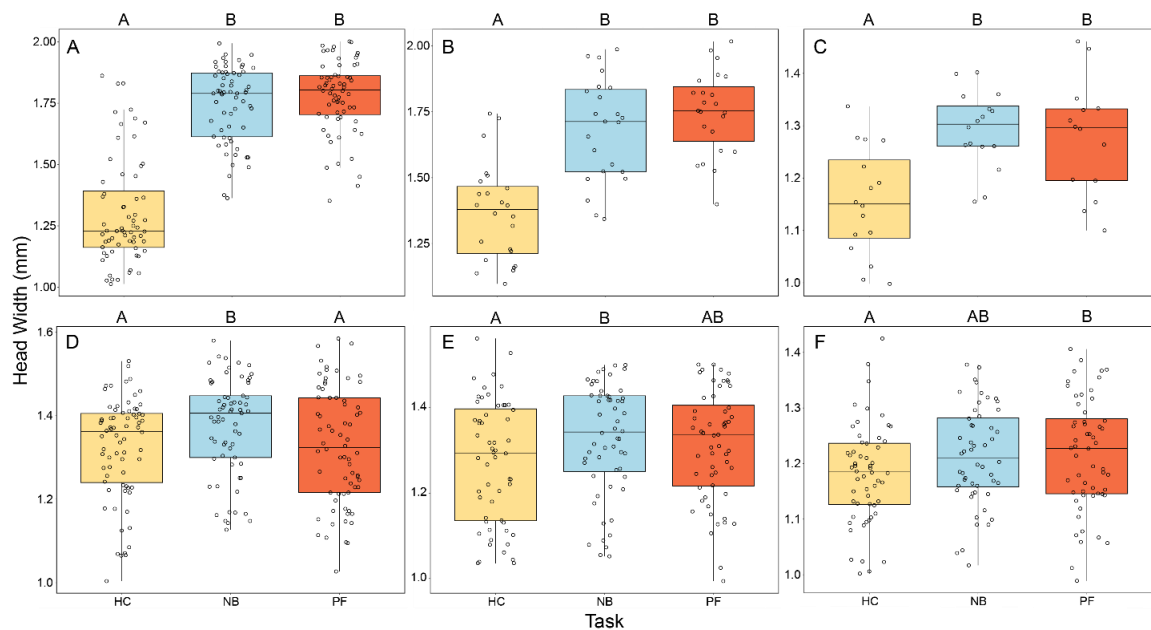
Figure 1.2 Boxplots depicting the association between task (HC = honeydew collecting, NB = nest building, and PF = protein foraging) and head width for each of three mound-building *Formica* species: *F. obscuriventris* (A), *F. aserva* (B), *F. dakotensis* (C) and three subterranean *Formica* species: *F. podzolica* (D), *F. glacialis* (E), and *F. neoclara* (F). Letters are placed above each box to signify whether there is a significant difference between the head widths of workers from each task based on Tukey's range post hoc test.

Effect of Degree of Within-Colony Size Variation on the Association between Size and Task

The 48 colonies included in our phylogenetic independent contrast exhibited a range of worker size variance (0.002 - 0.117 mm). As intra-colony worker size variation increased, the association between worker size and task strengthened, when comparing certain tasks (Fig. 1.3). This pattern was particularly strong when comparing honeydew collectors to nest builders (R^2=0.24, p<0.0001) and protein foragers (R^2=0.33, p<0.0001) (Fig. 1.3A-B). However, an increase in worker size variation did not affect the strength of the size-task association when comparing nest builders and protein foragers (R^2=0.050, p=0.074) (Fig. 1.3C).
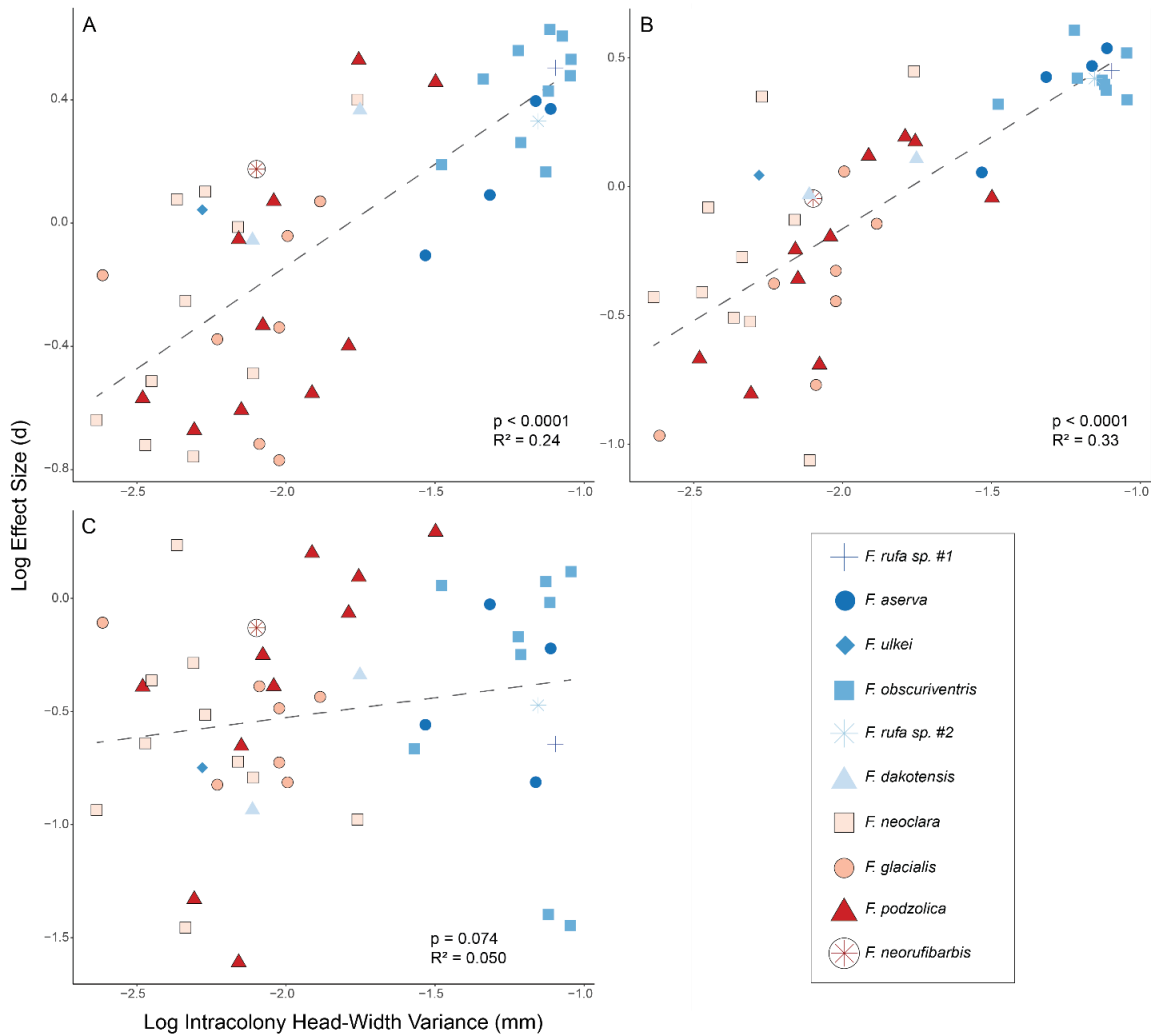
Figure 1.3: The relationship between effect size of the size-task association within each colony (Cohen's d, log-transformed) and intracolony worker size variance (log-transformed) for each task comparison: honeydew collectors compared to nest builders (A), honeydew collectors compared to protein foragers (B), and nest builders compared to protein foragers (C). Regression lines are shown for each task comparison (dashed lines). Colonies from subterranean species are represented by red shapes (outlined in black) and colonies from mound-building species are represented by blue shapes (no outline). Within each plot, the p and R^2 values are based on results from phylogenetic independent contrasts.

Discussion

We provide strong support for a form of morphological polyethism operating in *Formica* ants. In addition to exhibiting high task fidelity, *Formica* ants partition non-reproductive tasks on the basis of worker size. Other social insects that lack discrete

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morphological worker subcastes utilize variation in size among workers in their task

partitioning strategies (stingless bees: Grüter et al. 2012; Grüter et al. 2017; fire ants:

Tschinkel et al. 2003; Tschinkel 2013; Wilson 1978; bumble bees: Richards 1946;

Cumber 1949; Brian 1952; Jandt & Dornhaus 2009; sweat bees: Spradbery 1972; Breed

et al. 1978; wasps: O'Donnell & Jeanne 1995). Researchers often assume that insect

societies lacking discrete morphological worker subcastes utilize temporal polyethism, a

task partitioning strategy wherein individuals switch tasks as they age (reviewed in

Robinson 1992; Goldsby et al. 2012). We provide evidence to suggest that morphological

polyethism is potentially much more common in these insect societies than previously

expected, whether alongside or in the absence of temporal polyethism.

Though we suspect that temporal polyethism is not shaping the allocation of the

external tasks we observed in *Formica*, we cannot rule it out. We conducted our

observations over brief periods, and task switching may occur over longer timescales as

workers age. Temporal and morphological polyethism operate alongside each other in

some species (e.g., Seid & Traniello 2006; Camargo et al. 2007; Muscedere et al. 2009),

and it's possible that a similar strategy exists in *Formica*. For example, for tasks where

there is no observable difference in size, age might contribute to task fidelity. In mound-

building *Formica*, for example, large workers may specialize in nest building and protein

foraging at different ages. Additionally, we have not investigated within-nest tasks in the

present study, because the necessary destructive sampling would have undermined our

mark-recapture approach. However, small stingless bee workers stay within the nest

longer than their larger sisters (Hammel et al. 2016) and, in many ant species, workers

change from within-nest tasks to external tasks as they age (e.g., Retana & Cerdá 1990; Vieira et al. 2010). *Formica* workers may have a similar task trajectory. Although we observed continuing task fidelity in two colonies up to a month after our initial markings, a longer-term study is needed to determine whether temporal polyethism operates alongside morphological polyethism in *Formica*.

Finding high levels of task fidelity reveals that *Formica* colonies employ a successful task allocation system. Our comparison of task allocation across species and colonies that differ in the magnitude of worker size variance suggests that there may be two alternative strategies operating within *Formica*. When size variance is high, workers are more likely to carry out tasks according to their size (Fig. 1.3). However, size is not the only factor operating in this system, since we find task fidelity, but no size difference, among nest building and protein foraging workers (Fig. 1.2A-C; Fig. 1.3C). When there is very little size variance, workers still carry out tasks according to their size, but there is far less consistency between colonies (Fig. 1.2D-F; Fig. S1.2). This indicates the possibility that their body size and, potentially, associated shape may predispose ants to take on different tasks, but that the reaction norms may vary according to other factors (e.g., genetics, social or abiotic environment). Thus, we see two extremes: high task fidelity and size differences distinguishing honeydew collectors from nest builders and protein foragers consistently in species with large worker size variance, and high task fidelity with less distinct and inconsistent size differences in colonies with low worker size variance.

We do not yet know whether the degree of size variance is a by-product of differences in genetic background or development, or whether selection shapes the distribution of worker sizes. Notably, the three mound-building species are more closely related to each other than to any of the subterranean species (Romiguier et al. 2018; Fig. S1.6). However, a phylogenetic independent contrast suggests that even when correcting for phylogeny, the degree of intracolony size variation among workers significantly impacts the association between size and task across *Formica* (Fig. 1.3). Additionally, although the mound-building species generally possess greater worker size variance than subterranean species, there are some cases in which mound-building species possess similar (e.g., *F. dakotensis*, Fig. S1.4C) or lower (e.g., *F. ulkei*, Fig. S1.5D) worker size variance than some subterranean species. As mentioned previously, the ecology and life histories of mound-building and subterranean species differ, but the two groups often exist alongside each other within the same habitats. Clearly, there is more to learn about the factors shaping both worker size variance and task allocation strategy in this widespread, ecologically important genus.

Within-colony task dynamics will be important to investigate in future studies in order to comprehensively understand the degree of worker flexibility and the presence or absence of temporal polyethism in this system. Although we report high task fidelity across short timescales, protein foragers are much more likely to switch tasks than either honeydew collectors or nest builders. This observation might be explained by the relative needs of *Formica* colonies. Bernstein (1976) estimated that protein comprised only about 10% of a typical *Formica* colony's diet, suggesting that protein may be in lower demand

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than honeydew, for example. Additionally, as Dussutour and Simpson (2008) demonstrate, ants regulate their foraging strategies based on the number of developing brood and available resources. This may cause workers to switch between protein foraging and other tasks as the needs of the colony change. Such flexibility may be beneficial to the colony during seasonal changes in protein availability. Additionally, low predictability or reliability of protein sources may lead to opportunistic protein foraging when workers encounter prey items while performing their usual tasks. Further, our ability to detect protein foraging was lower than the other tasks because it occurs over a large area and successful foraging trips are likely rare. Thus, by restricting our mark-recapture efforts to individuals that were returning to the nest after successfully collecting insect prey, our results may underestimate the effort and task fidelity of protein foragers. It is important to note that the task dynamics depicted in Figure 1.1 are summed across multiple colonies of several different mound-building and subterranean *Formica* species. Even so, our results suggest that subterranean species, which tend to possess more uniformly sized workers, have a significantly higher rate of task-switching than mound-building species.

More generally, all insect societies utilizing morphological polyethism likely share some aspects of developmental processes, evolutionary history, and limitations on worker behavioral flexibility because they all utilize body size variation (Wheeler 1991). Here, we consider some differences between species with and without discrete morphological worker subcastes that may have important implications for colony performance and species longevity. Worker size in all insect societies is shaped by

developmental conditions (reviewed in Wills et al. 2018), including the temperature and

nutritional quality (Parker & Johnston 2006), social environment (Purcell et al. 2012;

Shpigler et al. 2013), genetics (Hughes et al. 2003; Jaffe et al. 2007; Schwander et al.

2005), and development time (Purcell & Chapuisat 2012; Shpigler et al. 2013), the latter

of which may have an epigenetic basis (Alvarado et al. 2015). The production of

morphological subcastes, however, relies on additional complex hormonal pathways and

genetic networks that regulate differential growth patterns of individual tissues in

developing workers (reviewed in Trible & Kronauer 2017). From an evolutionary

perspective, workers of different sizes may be better at performing different tasks, such

that the maintenance of size variability reinforces task specialization and enhances colony

efficiency (Oster & Wilson 1979; Chittka & Muller 2009). Past studies propose that an

additional evolutionary step, in which exaggerated morphologies are favored by natural

selection over intermediate morphologies, led to insect societies with discrete

morphological subcastes (Wilson 1953; Emlen & Nijhout 2000; reviewed in Trible &

Kronauer 2017). However, we should not infer that all social insects with size variable

workers are on an evolutionary trajectory toward the development of discrete subcastes.

On the contrary, we speculate that species with continuous size variation and size-based

task partitioning might achieve higher fitness than species with morphological subcastes

under some conditions. Previous work, though limited, suggests that workers in insect

societies with discrete morphological subcastes are less behaviorally flexible than in

those without them. For example, major *Pheidole* workers are less successful at raising

brood compared to minor workers (e.g. Mertl & Traniello 2009), while large

Tetragonisca angustula workers are capable of performing all the tasks that small workers usually perform (Hammel et al. 2016). Differences in restrictions imposed by alternative task allocation strategies are important to consider, as they may impact the resilience of the species that use them in increasingly unpredictable environments (Fisher et al. 2019).

We provide strong evidence that *Formica* ant workers use a size-based task allocation strategy. Among eusocial Hymenoptera, the presence of discrete morphological worker subcastes is relatively uncommon, occurring only in 15% of ant genera (Oster & Wilson 1979). Thus, a primarily size-based form of morphological polyethism may be far more common than suggested by the literature. Morphological polyethism should not be ruled out in social insects known to use temporal polyethism, as the two strategies have been found to operate alongside each other in some species with morphological subcastes. We further identify several likely developmental, evolutionary, and behavioral distinctions between workers from species with and without morphological subcastes and argue that these should be considered in future studies. More comparative analyses will give us a better understanding of how variation within and across species impacts the robustness and flexibility of different task allocation strategies.

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Chapter II: Worker size affects task proficiency in ants utilizing a size-based task partitioning strategy

Introduction

The ability of insect societies to partition tasks, which is accomplished without any form of central control (Gordon 1996), is believed to contribute greatly to their ecological success (Oster & Wilson 1978). Task specialization is thought to increase the efficiency of societies by enabling individuals to effectively learn skills associated with a small suite of tasks and avoid costs associated with switching tasks (Chittka & Muller 2009). Indeed, task-switching is associated with significantly longer intervals between tasks (Leighton et al. 2017) and can lead to poor attempts by workers at performing tasks for which they are ill-suited (Ushitani et al. 2016). A combination of these costs may lead to decreased colony efficiency and may even contribute to colony collapse (Perry et al.

2015). Although task specialization is thought to reduce task-switching costs, it may also reduce behavioral flexibility. For example, *Pheidole* major workers, which possess disproportionately large heads and specialize in colony defense, perform poorly at brood care tasks usually performed by the much smaller minor workers (Mertl & Traniello 2009). Ultimately, it is unclear whether task specialization leads to higher colony performance, especially in times of stress, when workers' ability to expand their task repertoires may be most advantageous.

Worker variation theoretically facilitates task specialization through matching individual workers to tasks that they perform well or for which they have lower internal thresholds for responding to task-related stimuli ('response thresholds') (Beshers & Fewell 2001). Task specialization is often associated with variation in worker morphology (reviewed in Oster & Wilson 1978), physiology (e.g., Spaethe et al. 2007, Wittwer & Elgar 2018), or genetics (reviewed in Oldroyd & Fewell 2007), which may act in conjunction (e.g., Schwander et al. 2005, Spaethe et al. 2007, Wittwer & Elgar 2018). Despite the frequent association between worker diversity and task specialization, empirical evidence of any adaptive benefits, including task proficiency, of worker diversity is inconclusive. For example, diversity in morphology, genetics, and behavioral response thresholds is associated with more efficient brood production and higher survival rates in some social insects (*Formica*: Billick 2002, Billick & Carter 2007; *Apis mellifera*: Mattila & Seeley 2007; *Temnothorax longispinosus*: Modlmeier & Foitzik 2011), but not in others (*Bombus*: Cnaani & Hefetz 1994, Jandt & Dornhaus 2014; *Temnothorax nylanderi*: Colin et al. 2017, Honorio et al. 2020). Genetic diversity is

generally positively correlated with several metrics of colony fitness (reviewed in Oldroyd & Fewell 2007), but this is not always apparent (Rosset et al. 2005). Assessments of colony fitness are often used as indirect tests of whether worker diversity translates to task proficiency. Few studies have attempted to assess task proficiency directly, and these have also yielded mixed results. For example, Spaethe & Weidenmuller (2002) found that large bumble bee workers, which more often perform foraging tasks than small workers, are more efficient nectar foragers. In contrast, Dornhaus (2008) found that *Temnothorax nylanderi* task specialists are not any more efficient at the tasks they typically perform compared to other workers. At the colony level, other studies suggest that decreasing worker morphological or genetic diversity has little to no effect on task performance or efficiency (Colin et al. 2017; Wilson 1983, Rosset et al. 2005).

There are a few potential explanations for inconclusive results when investigating how colony and task performance are affected by worker diversity. First, task specialization may not yield greater task efficiency in all insect societies. In some cases, the degree of task specialization may simply be a byproduct of colony characteristics, such as size or life history, the need for a task relative to the number of workers available to perform it, or differences in individual characteristics, such as response threshold (Jeanson et al. 2007). None of these necessarily affect how well-suited a task specialist is to perform a given task. Second, experiments that can effectively assess task proficiency are inherently difficult to design. Unexpected behavioral changes in workers, such as an increased individual activity level (e.g., Wilson 1983) in laboratory conditions, can make

evaluating the effects of experimental manipulation on colony and individual task performance difficult to measure. Measuring colony and task efficiency in natural settings is also challenging (but see Honorio et al. 2020), because it is difficult to isolate worker diversity from confounding factors, such as colony size, an important predictor of colony fitness (e.g., Rosset et al. 2005). A third reason, perhaps the easiest to address, is that many studies fail to place the diversity metrics and degree of variation in the proper context. It is important to consider what worker characteristics contribute to task specialization before attempting to assess whether variation in those characteristics affects colony and individual task proficiency. For example, worker size is associated with task specialization in one species of *Temnothorax* (Herbers & Cunningham 1983), but it appears to be very plastic in most others (Pinter-Wollman et al. 2012). Additionally, *Temnothorax* colonies generally exhibit little worker size variation, but substantial worker behavioral variation (Modlmeier & Foitzik 2011), suggesting that behavioral variation may be more important for task specialization in this genus. Indeed, worker size diversity in *Temnothorax* appears to contribute little to colony fitness (Modlmeier & Foitzik 2011, Colin et al. 2017, Honorio et al. 2020, Honorio et al. 2021) and task proficiency (Colin et al. 2017), while variation in aggression is positively correlated with colony fitness (Modlmeier & Foitzik 2011). Thus, researchers must consider the degree of each form of worker variation and its relevance to a species' task partitioning strategy when assessing how division of labor contributes to colony fitness or task proficiency. A greater emphasis on comparative studies that include species with

varying degrees of worker diversity will also elucidate how and when task specialization may provide an adaptive benefit.

In this study, we aim to understand whether size diversity in an ant genus that utilizes a size-based task partitioning strategy confers benefits via task proficiency. *Formica* ant colonies are composed of workers that are continuously variable in size and lack discrete morphological worker subcastes (Tawdros et al. 2020). Previous work has shown that individual *Formica* workers are highly consistent in the tasks they perform in the field and that this task specialization is linked to worker body size (West & Purcell 2020). For example, in nature, small *Formica* workers specialize in honeydew collection, while some large workers specialize in nest construction. We utilized three *Formica* species that differ in the degree of intracolonial size variation to determine whether worker size is associated with task proficiency. We assembled groups composed of only small, only large, and a mix of small and large workers, challenged them with two different tasks (sugar-water collection and nest material movement) in a laboratory setting, and measured their performance. We address four main objectives: 1) assess the performance of groups of *Formica* workers of different average sizes, 2) estimate individual-level efficiency within groups by monitoring activity levels, 3) determine whether the degree of intracolony size variance affects differences in task proficiency, and 4) assess whether small and large workers exhibit task preferences. If *Formica*'s size-based task partitioning strategy confers fitness benefits via task proficiency, we would expect small workers to excel at honeydew collection and large workers to excel at nest construction, in line with the tasks that they typically perform in nature. Further, we would expect

differences in task proficiency to be greater in species with higher intracolonial size variance.

Materials and Methods

In June - July 2018, we collected workers from six colonies each of two mound-building species of *Formica* (*F. obscuriventris* and *F. ulkei*) in Fish Creek Provincial Park, Calgary, Alberta, Canada. In October – November 2018, we collected workers from six colonies of a third mound-building *Formica* species, *F. integroides*, in San Bernardino National Forest, Big Bear, California, USA. The three species differ in the degree of intracolony worker size variance. Based on head width measurements, which are a good proxy for overall body size (Tawdros et al. 2020), *F. obscuriventris* workers are highly variable in size (variance = 0.09), *F. integroides* workers are moderately variable in size (variance = 0.07), and *F. ulkei* workers are relatively uniform in size (variance = 0.02).

Ant Collection and Laboratory Setup

We collected approximately 200 workers of variable size from each field colony by using a shovel to dig into the first few inches of the nest mound, disturbing the workers and shoveling them into a fluon-lined collection bin along with some of their nest material. Once enough nest material was collected, we then collected additional workers using soft forceps and by placing the shovel on top of the nest mound, allowing workers to crawl onto it, and shaking them into the bin. We did not collect queens or brood, as we wanted to minimize our disturbance of the field colonies.

We brought the collection bins back to the lab and allowed the workers to acclimate to laboratory conditions for several hours. Based on visual examination of the workers, we then split workers from each colony into three 50-worker groups composed of 1) large workers only, 2) small workers only, and 3) a mix of workers of variable size. After experimental trials, we measured the head-widths (as in West & Purcell 2020) of 15 workers from each group to confirm that they significantly differed in average worker size (Fig. S2.1).

We transferred each worker group into 23 x 23 x 10 cm artificial nest boxes, lined with fluon to prevent escape (Fig. 2.1A). These nest boxes were split into four compartments – one 23 x 7.6 cm "nest" compartment, in which we placed a 15 mL tube filled with water and plugged with cotton, and three 15.4 x 7.6 cm "task" compartments, in which we placed the task materials during the experimental trials (Fig. 2.1A). Workers were able to move freely between each task compartment and the nest compartment through passageways cut into shared walls (Fig. 2.1A). After placing workers in the nest boxes, we allowed them to acclimate overnight, before we challenged them with collection tasks the following morning.

Figure 2.1: An example of our laboratory setup. A) A 23 x 23 x 10 cm compartmentalized artificial nest box. Compartments are outlined in black and passageways are indicated with yellow rectangles and black arrows. The nest box contains a 15 mL tube filled with water and plugged with cotton (bottom "nest" compartment), nest material in a weigh tray (upper middle "task" compartment), and sugar-water in a 1.5 mL tube with a cotton wick (upper left "task" compartment). B) Three artificial nest boxes containing different worker groups are shown side-by-side. Gopro™ Session 5 cameras were mounted directly above each nest box to collect video footage throughout each experimental trial.

Task Presentation

We presented each worker group with two tasks: nest material collection and sugar-water collection (Fig. 2.1A). For the nest material collection task, we provided each worker group with approximately 2.5 g of nest material collected from their own nest mounds in an aluminum weigh tray. Nest material was composed of a mixture of dirt,

dried grass, pine needles, and wood chips. For the sugar-water collection task, we provided each colony fragment a 1.7 mL tube filled with 500 µL of a 50% sugar-water solution (3 : 2.4 : 0.6 distilled water : powdered sugar (sucrose) : powdered melezitose). We included melezitose in the sugar-water mixture because it is a trisaccharide often found in aphid honeydew secretions and aphid-tending ants prefer it to other sugars (Kiss 1981; Völkl et al. 1999; Fischer & Shingleton 2001). To minimize evaporative loss, we filed the tip off of each 1.7 mL tube to expose a narrow opening that we plugged with cotton before adding the sugar-water solution and closing the cap.

Over three days, we first tested each task independently (days 1-2) and then both simultaneously (day 3). On each day, we recorded the weights of task materials hourly over an eight-hour period. To account for evaporative loss from the sugar-water and nest material, we prepared control task materials, as above, on each day of the experiment, placed them on the bench next to the artificial nest boxes throughout the experiment, and weighed them each hour alongside the treatment materials. Control nest material did not experience substantial evaporative loss, but control sugar-water did. Thus, we subtracted the amount of evaporative loss of the control sugar-water from the hourly weight of the sugar-water provided to each treatment group for all analyses.

The order in which we presented the tasks on the first two days was randomized for each field colony. The placement of the task materials in one of the three task compartments was also randomized. We challenged all three colony fragments made from a single field colony simultaneously. For *F. obscuriventris* and *F. ulkei*, collected in Alberta, we tested workers originating from one field colony of each species on the same

days. For *F. integroides*, collected in California, we tested up to two field colonies simultaneously. Both lab spaces are windowless and were illuminated solely by artificial overhead lights, decreasing the possibility that natural or artificial light sources contributed to sensory bias toward any nest box compartment. The overhead lights remained on throughout the experiment, to ensure consistent conditions and allow for video recording.

Video Recording

We mounted GoPro[TM] Session 5 cameras above the center of every nest box to record video of each worker group throughout the eight-hour treatments (Fig. 2.1B). For each worker group, we used manual counters to record the number of times a worker passed into and out of each task compartment during the first hour of video (162 hours total). For the remaining seven hours of each treatment, we counted workers that passed into and out of each task compartment for ten minutes within the last 20 minutes of each hour (189 hours total), when possible. In some cases (n = 10), the last 20 minutes of the hour were not recorded due to SD card errors or storage limits, so we used the last 10 minutes of available video for those hours. To assess the overall activity level of each worker group, we considered the number of "trips" as the sum of workers that moved into and out of each task compartment during the first hour. For the remaining seven hours, we estimated the number of trips by summing the number of workers that moved into and out of each task compartment during the 10 minute period that we watched and extrapolating this value across the remainder of the hour. Estimating the number of trips allowed us to approximate how many workers participated in each task via proximity to

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task materials, a metric often used in studies assessing task performance (e.g., Jandt & Dornhaus 2009, Fisher et al. 2022). This approximation enabled us to assess whether workers of different sizes exhibited task preferences.

Statistical Analyses

We performed all statistical analyses in R (V 3.5.0 or later, The R Foundation for Statistical Computing).

We created linear models for each species and task combination using the *lm* function. We used either the amount of nest material or sugar-water removed (g) or the amount of nest material or sugar-water removed per trip (g/trip) as the response variables. We started with five fixed effects: 1) the worker size treatment (small, mixed-size, and large), 2) the field colony, 3) the treatment hour, 4) the order in which the tasks were presented independently on the first two days (nest material or sugar-water first), and 5) the placement of the task material in the nest box compartments. We sequentially removed non-significant fixed effects to determine the best-fit model. From the best-fit models, we then performed post-hoc analyses using the *emmeans* function in the emmeans package (Lenth et al. 2022) to determine whether the amount of task material removed differed between treatment hours. All worker groups removed significantly more nest material from the weigh tray during the first two hours of the treatment than the remaining hours. There was often little nest material remaining in the weigh trays in the last six hours. Thus, all subsequent analyses include only the first two hours of data collected for the nest material task. Similarly, all worker groups removed significantly more sugar-water during the first hour than during the remaining hours. During these last

seven hours, the workers collected little sugar-water, suggesting that they became satiated after the first hour. Thus, all subsequent analyses included only the first hour of data collected for the sugar-water task.

After paring down our datasets, we removed the treatment hour from the fixed effects in our sugar-water models but continued to include it in our nest material models. As above, we then sequentially removed non-significant fixed effects to determine the best fit models for each species and task combination. To assess differences in the overall performance of each worker group, we used the *emmeans* function in the emmeans package (Lenth et al. 2022) to perform post-hoc analyses on the best-fit models with the amount of nest material or sugar-water removed (g) as the response variable. To assess differences in the relative efficiency of each worker group, we similarly performed post-hoc analyses on the best-fit models with the amount of nest material or sugar-water removed per trip (g/trip) as the response variable.

Across all three species, we tested whether our estimate of activity level was a good predictor of the amount of work performed. For this, we performed a linear regression for each worker group and task combination using the purrr package (Henry & Wickham 2022). For each linear regression, the number of workers that moved into and out of each task compartment (number of trips) was the fixed effect, and the amount of nest material or sugar-water removed was the response variable.

To assess whether the degree of intracolony size variance within and across species affected the differences in task performance, we created linear models for each treatment group comparison (large vs. small, mixed-size vs. small, and large vs. mixed

size) using the *lm* function. For these models, intracolony head-width variance was the fixed effect, and the difference in the amount of task material removed for each treatment group comparison was the response variable.

To assess whether differently sized workers exhibited a task preference, we used the *chisq.test* function to carry out chi-square analyses comparing the average number of trips for each task compartment (sugar-water and nest material) during the first hour of day three of laboratory trials, when we presented both tasks together. For each species and worker group combination, we measured whether the observed number of trips for each task compartment significantly differed from an equal number of trips for each compartment, as we would expect in the absence of a task preference.

Results

Group Performance

When we presented tasks separately, there were no significant differences in the amount of sugar-water or nest material removed by worker groups across all three species (sugar-water: Fig. 2.2A-C, nest material: Fig. 2.2D-F). When we presented tasks together, groups of small *F. obscuriventris* workers removed significantly less nest material than groups of large ($t_{28} = 3.02$, $p = 0.01$) and mixed-size ($t_{28} = 3.01$, $p = 0.02$) workers (Fig. 2.2G). Similarly, groups of small *F. integroides* workers removed significantly less nest material than groups of large workers ($t_{25} = 3.00$, $p = 0.02$, Fig. 2.2H). There was no difference in the amount of nest material removed among worker groups in the least size-variable species, *F. ulkei* (Fig. 2.2C, F, I). Additionally, there was no difference in the

amount of sugar-water removed by worker groups across all three species (Fig. S2.3A-

C).
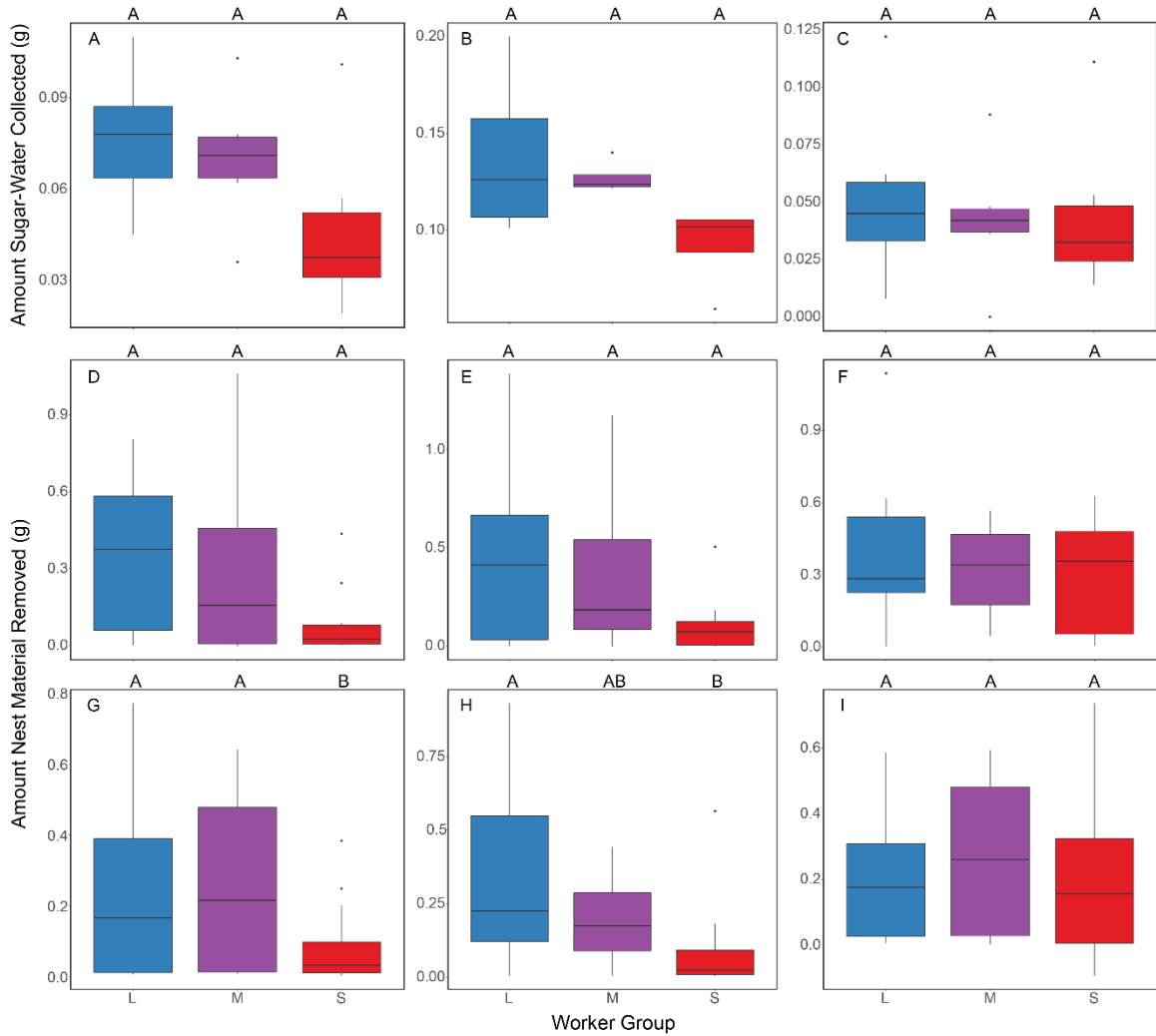
Figure 2.2: Boxplots depicting the amount of sugar-water collected (A-C) and nest material removed (D-I) by groups of large (L), mixed-size (M), and small (S) workers of *F. obscuriventris* (left), *F. integroides* (middle), and *F. ulkei* (right) when we presented tasks separately (A-F) and together (G-I). Different letters above each box represent significant differences between groups. Groups of large workers are shown in blue, groups of mixed-size workers are shown in purple, and groups of small workers are shown in red. Boxes represent the middle 50% of the data, horizontal lines within boxes represent median values, and outliers are represented by points.

Estimate of Activity Level

If our estimate of activity level is a good predictor of the amount of work performed, we would expect a positive correlation between the number of trips and the amount of task material removed. However, when we presented tasks separately (days 1 and 2), there was no correlation between activity level and the amount of sugar-water removed for any worker group across species (Fig. S2.2A). In contrast, there was a positive correlation between activity level and nest material removal for groups of large (p = 0.02) and mixed-size (p = 0.02), but not for groups of small (p = 0.6) workers (Fig. S2.2B). When we presented tasks together (day 3), there was still no correlation between activity level and the amount of sugar-water removed, except for a weakly positive correlation for groups of mixed-size workers (p = 0.04, Fig. S2.2C). However, there was a positive correlation between activity level and nest material removal for all worker groups when we presented tasks together (p < 0.001, Fig. S2.2D).

Individual Task Proficiency

When we presented tasks separately, groups of small *F. obscuriventris* workers removed significantly less nest material per trip than groups of large (t_{21} = 3.35, p = 0.008) and mixed-size (t_{21} = 2.58, p = 0.04) workers (Fig. 2.3A). In contrast, there were no significant differences in the amount of nest material removed per trip among worker groups in either *F. integroides* (Fig. 2.3B) or *F. ulkei* (Fig. 2.3C).

When we presented tasks together, groups of small *F. obscuriventris* workers removed significantly less nest material per trip than groups of mixed-size workers (t_{28} = 3.42, p = 0.005, Fig. 2.3D). Similarly, groups of small *F. integroides* workers removed

significantly less nest material per trip than groups of large workers ($t_{25} = 2.97$, $p = 0.02$, Fig. 2.3E). There was no difference in the amount of nest material removed per trip among worker groups of *F. ulkei* (Fig. 2.3F).

There was no difference in the amount of sugar-water removed per trip among worker groups when we presented sugar-water independently and alongside nest material (data not shown). As mentioned above, the number of trips was not correlated with the amount of sugar-water collected (Fig. S2.2). Thus, the amount of sugar-water collected per trip is not likely to be informative for assessing worker-level efficiency for this task.
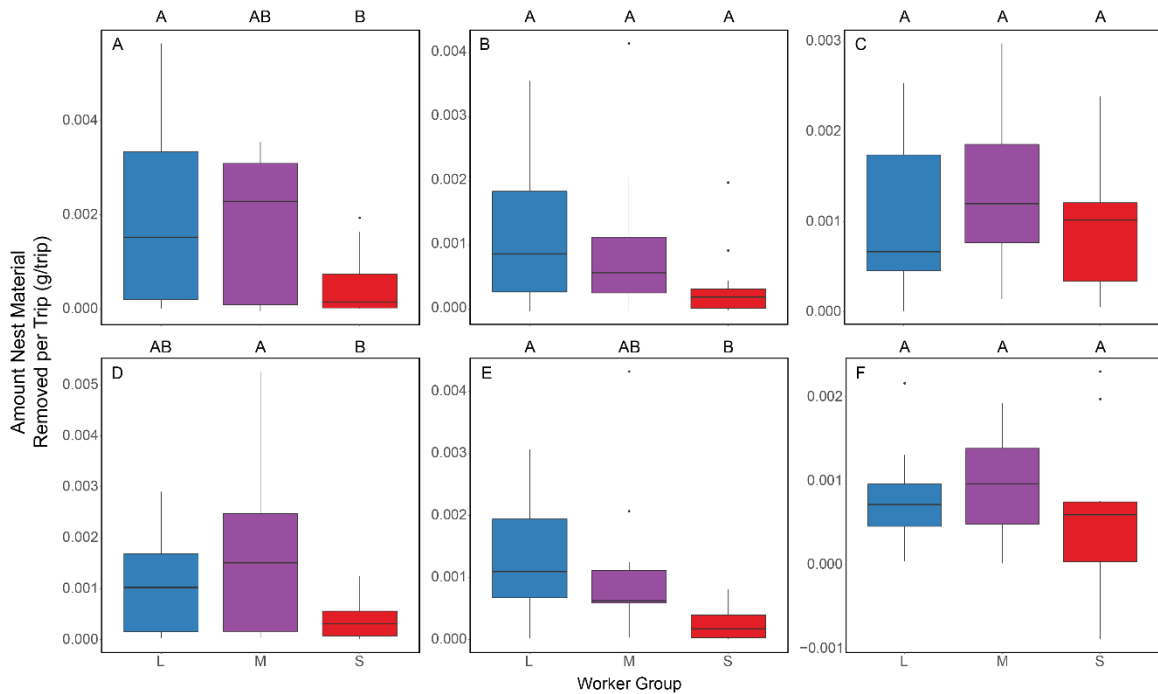
Figure 2.3: Boxplots depicting the amount of nest material removed per trip by groups of large (L), mixed-size (M), and small (S) workers of *F. obscuriventris* (left), *F. integroides* (middle), and *F. ulkei* (right) when nest material was presented independently (A-C) and alongside sugar-water (D-F). Different letters above each box represent significant differences between groups. Boxes represent the middle 50% of the data, horizontal lines within boxes represent median values, and outliers are represented by points.

Effect of Intracolony Size Variance on Size-Selected Group Performance

The difference in the amount of nest material removed by groups of small workers compared to groups of large and mixed-size workers was significantly correlated with intracolony size variance, both when the nest material was presented independently (Fig. 2.4A-B) and alongside sugar-water (Fig. 2.4D-E). There was no such correlation when we compared large and mixed-size groups (Fig. 2.4C, F). Across all three species, the degree of intracolony size variance did not significantly affect the difference in the amount of sugar-water removed by worker groups (Fig. S2.4).
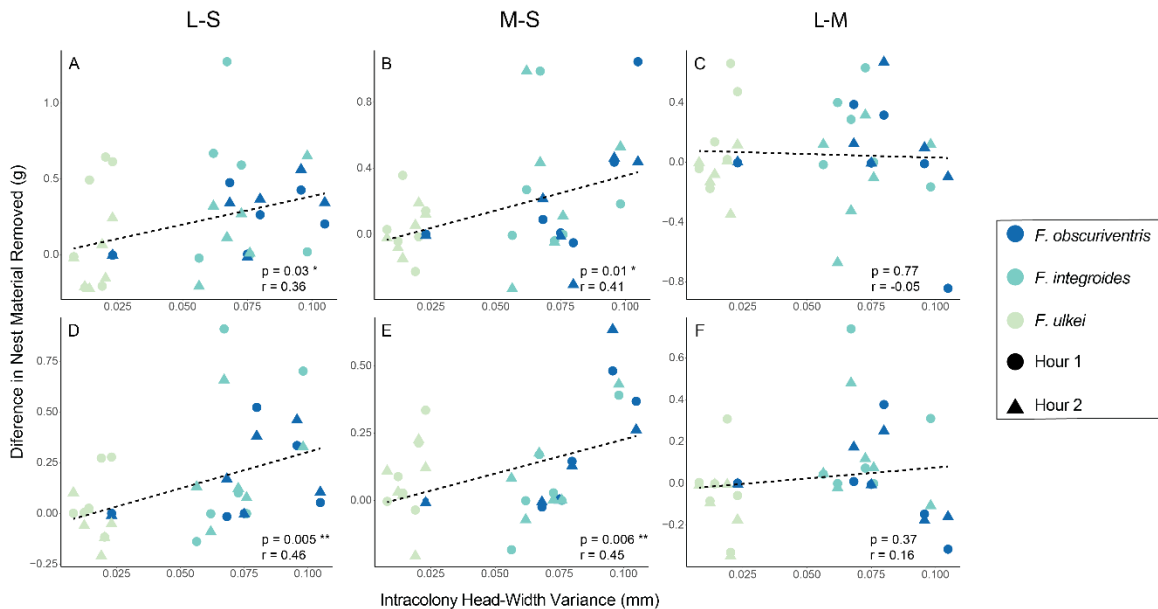
Figure 2.4: The relationship between the amount of intracolony worker size variance and the difference in performance by size-selected worker groups at nest material removal for three *Formica* species, when we presented tasks separately (A-C) and together (D-F). The difference in performance between groups composed of large and small workers (L-S), mixed-size and small workers (M-S), and large and mixed-size workers (L-M) are shown in left, middle, and right panels, respectively.

Task Preference

When we presented tasks together (day 3), all worker groups of all three species either exhibited a preference for the nest material compartment over the sugar-water compartment or no preference at all during the first hour of the experiment, based on the average number of trips for each task compartment. More specifically, groups of small *F. obscuriventris* workers ($p = 0.004$, Fig. 2.5A), all worker groups of *F. integroides* ($p < 0.001$, Fig. 2.5B), and groups of mixed-size ($p = 0.02$) and small ($p < 0.001$) *F. ulkei* workers (Fig. 2.5C) exhibited a significantly higher activity level in the nest material compartment than the sugar-water compartment. In contrast, groups of large and mixed-size *F. obscuriventris* workers (Fig. 2.5A) and large *F. ulkei* workers (Fig. 2.5C) did not exhibit a significant preference for either task.
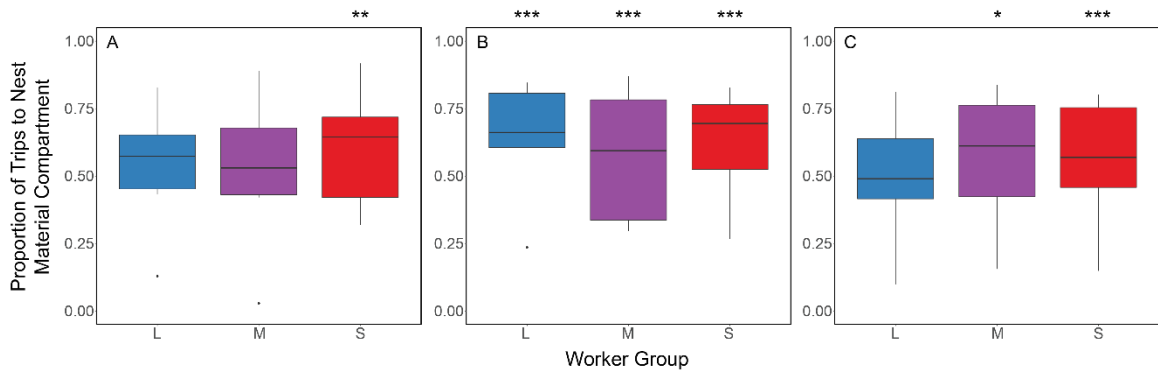
Figure 2.5: The proportion of total trips to the nest material compartment for each worker group of *F. obscuriventris* (A), *F. integroides* (B), and *F. ulkei* (C) during the first hour of day 3 of the experiment, when we presented tasks together. Above each boxplot, asterisks represent a significantly greater number of trips to the nest material compartment than the sugar-water compartment. "*" represents a p-value between 0.05 and 0.01, "**" represents a p-value between 0.01 and 0.001, and "***" represents a p-value less than 0.001. Boxes represent the middle 50% of the data, horizontal lines within boxes represent median values, and outliers are represented by points.

Discussion

In this comparative study, we utilized three species from a diverse ant genus with a size-based task partitioning strategy to assess whether worker size affects task proficiency. Ultimately, our results suggest that worker size diversity can alter task performance, both at the colony and individual level, but its effect is highly context- and species-dependent. This pattern was most apparent for laboratory tests of nest material removal. Within two *Formica* species with high intracolony worker size variance (*F. obscuriventris* and *F. integroides*), large workers removed significantly more nest material than small workers over the same amount of time and with similar estimated effort. For these same species, large workers performed equally well, if not better, than small workers at sugar-water collection. For a third *Formica* species with minimal intracolony worker size variance, large and small workers performed equally well at both tasks. We discuss our species-, group-, and individual-level results and their possible explanations, given what we know about task allocation in *Formica* from previous studies, in detail below.

Broadly, cross-species comparisons suggest that the greater the variation in body size among workers, the more likely it is that average worker size will affect group-level task performance. As intracolony worker size variation increases, so do the differences in the amount of nest material removed by groups composed of workers of different sizes (Fig. 2.4). When intracolony size variation is great, groups of large and mixed-size workers perform much better at nest material removal than groups of small workers. In contrast, the degree of intracolony worker size variance did not significantly affect the

differences in the amount of sugar-water collected by different treatment groups (Fig. S2.4). Interestingly, *Formica* intracolony worker size variation also significantly affects our ability to predict a worker's task given its body size, suggesting that species and colonies with greater worker size variation rely more heavily on a size-based task partitioning strategy (West & Purcell 2020). Together, these results suggest that size diversity facilitates task specialization, and, as size variation increases, differences in worker size are also more likely to affect task proficiency.

At the group level, we find similar patterns. More specifically, when intracolony worker size variation is substantial (*F. obscuriventris* and *F. integroides*), groups composed of large and mixed-size workers perform better at nest material removal and equally well, if not better, at sugar-water collection than those composed of only small workers. When we presented tasks separately, differences in task performance between worker groups are marginally non-significant, which might be explained by the relatively low power of our analysis due to small sample size (per species). When we presented tasks together, differences in nest material removal between worker groups were statistically significant, although all worker groups performed equally well at sugar-water collection. When intracolony worker size variation is minimal (*F. ulkei*), groups composed of large, mixed-size, and small workers performed equally well at both tasks, regardless of whether we presented tasks separately or together.

The result that groups of large workers, which specialize in nest building in nature (West & Purcell 2020), outperform groups of small workers at nest material removal aligns well with our expectations. Further, at the individual level, large workers remove

significantly more nest material per estimated trip than small workers. Large workers'

greater proficiency at this task has a few potential explanations. First, the likelihood that

large workers had prior experience handling nest material has perhaps allowed them to

learn the skills necessary to succeed at this task (Chittka & Muller 2009). This experience

may have given them an advantage over small workers that may have been naïve to nest

building at the start of the laboratory experiment. Further, small workers may be ill-suited

for carrying nest material compared to large workers, just as large *Messor barbarus*

workers are more efficient than small workers at transporting seeds (Bernadou et al.

2016). Large ant workers may be especially well-suited to transporting large loads, as

evidence that harvester ant body size is strongly correlated with seed selection suggests

(Kaspari 1996). Indeed, the nest material provided to workers in this experiment was

often quite heterogenous in size, and we noted that groups of small *F. obscuriventris* and

F. integroides workers appeared to leave behind large pieces of nest material in the

provided weigh trays most often, although we did not test this.

  The result that groups of small workers did not outperform groups of large

workers at sugar-water collection, is unexpected. Given that small *Formica* workers

specialize in honeydew collection in nature (West & Purcell 2020), we would expect

small workers to outperform large workers at sugar-water collection. Although

differences in the amount of sugar-water collected by groups of differently sized workers

were not statistically significant, we note that groups of large and mixed-size workers did

qualitatively perform better at this task than groups of small workers, at least for the two

most size-variable species (*F. obscuriventris* and *F. integroides*, Fig. 2.2A-B). The lack

of statistical significance may be the result of the relatively low power of our analyses due to small sample size (per species). Task performance based on worker body size is relatively understudied. However, previous work suggests that large bumble bee workers sometimes excel at tasks that small workers are more likely to perform. Small bumble bee workers generally carry out within-nest tasks (i.e., brood tending and nest maintenance), while large workers are more likely to leave the nest to forage (Cumber et al. 1949, Jandt & Dornhaus 2009). However, large workers seem to outperform small workers at many tasks, including foraging (Spaethe & Weidenmuller 2002) and brood rearing (Cnaani & Hefetz 1994).

If large workers outperform small workers, why do colonies continue to produce small workers? Several external and internal factors, such as environmental conditions and genetics, may contribute to the maintenance of worker size diversity in *Formica* colonies. Experimental manipulation of environmental conditions such as temperature (Molet et al. 2017), social environment (Purcell et al. 2012, Shpigler et al. 2013), and nutrition (Smith et al. 2008) influences the size of workers produced. A worker's genetic lineage is often associated with its body size (Hughes et al. 2003, Schwander et al. 2005). Further, epigenetic factors may influence developmental time (Alvarado et al. 2015), which ultimately influences body size (Purcell & Chapuisat 2012). Thus, variation in environmental conditions and food availability, in addition to a colony's genetic make-up, may constrain the size of workers produced at any given time.

Alternatively, if small workers are not simply a byproduct of developmental constraints imposed on social insect colonies, they may provide adaptive benefits that we

did not investigate in this study. For example, small workers, which require less food (Smith et al. 2008) and develop more quickly (Alvarado et al. 2015, Molet et al. 2017) than larger workers may be less costly to produce. Thus, it is possible that many small workers can be produced with the same amount of time and resources as a few large workers, and they may ultimately contribute more to colony productivity. Additionally, small *Formica* workers may provide advantages when resources are scarce. In bumble bees, small workers are more resistant to starvation than large workers (Couvillon & Dornhaus 2010), although this may be driven by the social environment present in incipient colonies (Costa et al. 2021). Small *Formica* workers may similarly enable their colonies to survive bouts of starvation in times of stress. Finally, small *Formica* workers may outperform large workers at tasks that we did not measure, such as brood care.

It may also be the case that our experimental design did not accurately assess honeydew collection tasks as they occur in nature. Indeed, collecting honeydew from sapsucking insects may involve multiple skills than are unnecessary when collecting sugar-water from a cotton wick, as it was presented in our experiments. For example, small workers may have an advantage over large workers when climbing trees and branches (Endlein & Federle 2015, Pattrick et al. 2018), interacting with sapsucking insects, or ingesting honeydew, depending on its composition, flow rate, and viscosity (Medan & Josens 2005). Additionally, although large workers were able to collect more sugar-water overall, all ants quickly became satiated within the first hour, and we may not have measured performance frequently enough to accurately assess collection efficiency. As our estimate of activity level (number of trips) rarely aligned with the

amount of sugar-water collected, we are unable to assess individual-level efficiency at this task. Large workers ultimately had more space in their crops to store collected sugar-water, but this does not necessarily mean that they fed at a higher rate. Thus, it is possible that small workers do outperform large workers at honeydew collection in nature, but our experimental design was incapable of detecting this.

Finally, when both tasks were presented together, all worker groups were either more active around the nest material than the sugar-water or equally active around both task materials. This may or may not be indicative of task preference. Although the number of trips into and out of the nest compartment was significantly correlated with the amount of nest material removed by large and mixed-size workers, this was not always the case for small workers. Further, due to limitations of our experimental design, it is difficult to disentangle attraction to the nest material, which most likely possessed each colony's odor, and task performance "effort." Proximity to a task material has often been used as a proxy for task performance (e.g., Jandt & Dornhaus 2009, Fisher et al. 2022). Although this may be appropriate in some cases, this does not necessarily mean that it provides an accurate estimate of if and when an individual is participating in a given task. We can see this clearly from the lack of correlation between the number of trips to the sugar-water compartment and the amount of sugar-water collected in this study. Alternatively, individual tracking of a subset of workers and their interaction with task materials would provide a more accurate estimate of what proportion of workers are actively participating in a task but would also severely limit plausible sample size.

Overall, this study suggests that, when size variation among workers is substantial, a size-based task partitioning strategy may enhance task efficiency. Large workers, which specialize in nest building in nature, outperformed small workers at this task in controlled laboratory experiments. However, it also supports the idea that task specialization does not always lead to task proficiency. Small *Formica* workers, which specialize in honeydew collection in nature, surprisingly did not outperform large workers at sugar-water collection in laboratory experiments. Further research is needed to determine if this is due to an imperfect experimental design or whether small *Formica* workers confer benefits, such as low production costs or proficiency at other tasks, that this study did not assess. Ultimately, both large and small workers can successfully perform both tasks, albeit with differing proficiencies. Thus, a size-based task partitioning strategy may take advantage of the relative abilities of a society's workers while also maintaining behavioral flexibility, which may be advantageous, particularly in times of stress.

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Chapter III: The genetic underpinnings of a size-based task partitioning strategy in socially polymorphic ants

Introduction

High genetic diversity in polygyne or polyandrous insect societies may counteract the proposed inclusive fitness benefits of group-living. Theoretically, cooperation among group members is most beneficial when those members are highly related (Hamilton 1964), which has often been used to explain the prevalence of haplodiploid insect societies, in particular (e.g., Trivers & Hare 1976, Foster et al. 2006). However, increasing evidence suggests that there are many benefits to high genetic diversity, including increased fitness and adaptability. A well-known example is the ability of genetically diverse insect colonies to overcome disease via social immunity (reviewed in Wilson-Rich et al. 2009). High genetic diversity may also increase a social insect

colony's ability to adapt to rapidly changing environmental conditions through enhanced thermoregulation (Jones et al. 2004) and foraging (Mattila & Seeley 2007, Wiernasz et al. 2008), which may each contribute to survival and reproduction (e.g., Mattila & Seeley 2007).

One potential explanation for many of the benefits associated with increased genetic diversity is enhanced division of labor (Oldroyd & Fewell 2007). Task specialization is often associated with worker morphology (e.g., size: Grüter et al. 2012, Tschinkel 2013) or physiology (e.g. response threshold to task related stimuli: Pankiw & Page Jr. 2000), both of which may be subject to genetic control. For example, distinct genetic lineages in polygyne (multi-queen) and polyandrous (multi-male) societies disproportionately contribute to workers of different morphological castes (Hughes et al. 2003, Rheindt et al. 2005), sizes (Schwander et al. 2005), and response thresholds (Calderone & Page Jr. 1991, Page Jr. et al. 1998). Thus, it follows that genetic diversity should also be correlated with the degree of task specialization. Indeed, in species that partition tasks based on age or behavioral response thresholds, several studies show a direct link between genetics, physiology, and the task(s) a worker performs (e.g., Julian & Fewell 2004, Jones et al. 2004, Chapman et al. 2007). Similarly, in some species with discrete morphological worker subcastes that specialize in different tasks, workers from the same caste and task group are more likely to have shared ancestry (Hughes et al. 2003, Rheindt et al. 2005). However, less is known about whether genetic task specialization and worker morphology are similarly linked in species with size-based task partitioning strategies.

Formica is an ant genus widespread across the Earth's northern hemisphere (Francoeur 1973). Most Formica species that have been studied thus far are socially polymorphic, meaning that some colonies are monogyne (single queen) while others are polygyne (Keller 1993, DeHeer & Herbers 2004, Rosset & Chapuisat 2007, Zahnd et al. 2021). Across species, a colony's social form is controlled by the 'social supergene,' a large non-recombining region on Chromosome 3 (Purcell et al. 2021). Additionally, some Formica species utilize a size-based task partitioning strategy, wherein differently sized workers specialize in different tasks (West & Purcell 2020), and, in some cases, task specialization is associated with increased task efficiency (Chapter II). In one species, F. selysi, a colony's social form plays a role in task allocation, such that worker size and task specialization are associated with genetic lineage in polygyne and polyandrous colonies (Schwander et al. 2005). However, these three factors were often independently associated, suggesting that there is not a direct link between genetics, size, and task in this species (Schwander et al. 2005). Even less is known about the interplay between worker size, task partitioning, and social form in other Formica species.

In this study, we assess the genetic underpinnings of a size-based task partitioning strategy in five socially polymorphic Formica species. Within each species, we determined the social form (monogyne vs. polygyne) of each colony and carried out further genetic analyses to address three aims: 1) Within polygyne colonies, we assessed whether there is an association between a worker's matriline and its size or task. 2) In comparing monogyne and polygyne colonies, we investigated whether genetic diversity

is associated with average worker size or intracolony variation. Finally, 3) within species, we identified genetic regions associated with worker size or task.

Materials and Methods

Sample Collection and Head Measurements

We collected ant workers from large, active colonies using a two-day mark-recapture protocol, as in West and Purcell (2020). In brief, we collected workers that we observed performing three different tasks: honeydew collection (HC), nest building (NB), and protein foraging (PF). We painted workers' abdomens with randomly assigned task-associated colors using a small dot of Testors® enamel paint and returned them to their nests. On a second day of observation, one to a few days later, we recaptured paint-marked workers, noted whether they performed the same or a different task, and placed them into a fluon-lined plastic bin. At the end of observation, we separated recaptured workers based on task and placed up to 15 workers per task into 15 mL tubes filled with 100% ethanol for preservation. We also collected up to 15 each of unmarked honeydew collectors, nest builders, and protein-foragers, observed performing those tasks for the first time on the second day of observation, into separate 15 mL tubes of 100% ethanol. Most recaptured workers (98.6%) performed the same task on both observation days (West & Purcell 2020).

For this study, we used workers from 28 colonies of 5 *Formica* species: *F. obscuriventris* (n = 6), *F. aserva* (n = 4), *F. glacialis* (n = 6), *F. podzolica* (n = 6), and *F. neoclara* (n = 6). For each colony, we measured the head widths of 12 workers, four workers for each of the three tasks. Of these, 180 were recaptured workers that

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consistently performed the same task (HC = 110, NB = 49, PF = 21), and 155 were

workers that were first observed performing that task on the second day of observations

(HC = 2, NB = 63, PF = 91). We measured the head widths to one-thousandth of a

millimeter of all 336 workers, as in West and Purcell (2020).

DNA Extraction, Sequencing, and Filtering

We extracted DNA from the head or head and thorax (depending on worker size)

of all 336 workers using the Qiagen DNeasy protocol for insect tissue, with the following

modifications: 1) we used freshly prepared 70% ethanol in place of Buffer AW2, 2) we

eluted DNA with 30 µL of double distilled H_2O instead of 200 µL of Buffer AE, and 3)

we used non-Qiagen spin-colums from BPI-Tech. We then carried out a high-density

restriction-site-associated DNA sequencing (ddRADseq) protocol to sequence fragments

of DNA across the genome (Brelsford et al. 2016). In brief, we digested DNA using MseI

and PstI restriction enzymes, ligated uniquely-barcoded adapters, and used a mix of

Agencourt AMPure paramagnetic beads and PEG/NaCl buffer to remove DNA fragments

shorter than 200 bp. To minimize PCR bias, we split each sample into four replicates

prior to initial PCR amplification. We then re-pooled replicates before an additional

round of PCR to close any single-stranded DNA fragments. We used gel electrophoresis

to assess the quality of PCR products, pooled viable samples, and sequenced them on the

Illumina Novaseq platform at the Novogene Corporation, Sacramento, CA, USA.

We demultiplexed samples using the process_radtags function from Stacks

(Catchen et al. 2013), organized samples by species, and aligned paired-end reads using

PEAR (Zhang et al. 2014). We aligned *F*. *obscuriventris* and *F*. *aserva* samples to a *F*.

exsecta reference genome (Lagunas-Robles & Brelsford, unpublished) and *F. glacialis*,

F. podzolica, and *F. neoclara* samples to a *F. selysi* reference genome (Brelsford et al.

2020) using BWA-MEM (Li 2013). We called single-nucleotide polymorphisms (SNPs)

using the mpileup command of BCFtools (Li et al. 2009, Li 2011, Danecek et al. 2021)

and applied quality filters using VCFtools (Danecek et al. 2011). Specifically, we only

retained SNPs with less than 20% missing data, minor allele count greater than or equal

to 3, and read depth greater than or equal to 7. We also removed SNPs with excessively

high read depths (95% percentile of each species), SNPs that altered the length of the

reference allele (indels), and all SNPs located on chromosome 3 to prevent the supergene

located on this chromosome that controls colony social form (single-queen vs. multi-

queen) from skewing further analyses. Further, we removed individuals with a mean read

depth less than 10 and with missingness greater than 50% from our analyses. After

filtering, retained SNPs had mean read depths of between 735 and 4840 per site, per

individual (Table S3.1).

Assessing Associations between Genetic Lineage, Task, and Size

We further pruned SNPs in high linkage disequilibrium from our dataset using the

--indep-pairwise function in PLINK (Chang et al. 2015). Using these pruned datasets, we

estimated parentage of individuals from each colony using COLONY (Jones & Wang

2010). When estimating the parentage of each colony, we allowed for polygamous

polygyny (multiple queens, multiply-mated) and monogamous polyandry (multiple

males, singly-mated). We also used pruned datasets to estimate pairwise relatedness

(Novel Estimator A) using PolyRelatedness V1.6 (Huang et al. 2016).

Analyzing polygyne colonies only, we generated linear mixed-effects models to assess the relationship between task and pairwise relatedness for each species using the *lmer* function in the lme4 package with the lmerTest modification. First, we set whether pairs of workers performed the same or different tasks as the fixed effect, pairwise relatedness as the response variable, and colony as a random effect. Then, to assess differences between each specific task comparison, we set task comparison as the fixed effect, pairwise-relatedness as the response variable, and colony as the random effect. We determined if the average pairwise relatedness differed among task comparisons using Tukey's range post hoc tests. Similarly, using the *lm* function, we generated linear models to assess whether there was an association between matriline and worker size in polygyne colonies for each species. In these models, matriline was the fixed effect and worker head width (as a proxy for body size) was the response variable. We again used Tukey's range post hoc tests to compare the average head width of workers of each matriline within polygyne colonies.

Assessing Associations between Social Form, Worker Size, and Intracolony Variance

For each species, we generated linear models using the *lm* function to assess whether monogyne and polygyne colonies differed in average worker size. For these models, social form (monogyne vs. polygyne) was the fixed effect and head width was the response variable. Additionally, we measured intracolony worker size variance using coefficients of variation, defined by Ferguson-Gow et al. (2014) as:

$$Coefficient\ of\ variation = 100 \left(\frac{\sigma\ worker\ head\ width}{\bar{x}\ worker\ head\ width} \right)$$

We then compared coefficients of variation between monogyne and polygyne colonies of each species with a Wilcoxon rank sum test, using the *wilcox.test* function. All statistical analyses were carried out in R version 3.5.2.

Genome-Wide Association Study

We performed four separate genome-wide association studies (GWAS) to assess whether any SNPs are associated with 1) worker size, 2) honeydew collection, 3) nest building, or 4) protein foraging. Starting from our raw datasets, we imputed missing data using BEAGLE (Browning & Browning 2016), removed all sites and individuals that did not pass our previously described filtering parameters, and carried out GWAS with GEMMA (Zhou & Stephens 2012), using the Wald test statistic. We adjusted p-values using the *p.adjust()* function in R based on a false discovery rate (Benjamini & Hochberg 1995) to identify size- or task-associated SNPs.

Results

Based on results from COLONY and our estimates of intracolony pairwise relatedness, 15 out of 28 colonies were polygyne (*F. obscuriventris* = 4, *F. glacialis* = 4, *F. podzolica* = 2, *F. neoclara* = 5). All four *F. aserva* colonies were monogyne. Thus, we did not proceed with analyses for *F. aserva*.

Associations between Genetic Lineage, Task, and Size

In *F. obscuriventris*, workers that performed the same task were more closely related than workers that performed different tasks ($F_{1,248} = 90.12$, $p < 0.001$). Specifically, honeydew collectors were more closely related to each other and nest

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builders than they were to protein foragers, nest builders were more closely related to each other and honeydew collectors than they were to protein foragers, and protein foragers were more closely related to each other than to either honeydew collectors or nest builders (Fig. 3.1A). According to analysis with COLONY, *F. obscuriventris* workers that performed the same task were siblings 97% of the time (full siblings = 63%, half siblings = 34%), while workers that performed different tasks were siblings only 53% of the time (full siblings = 21%, half siblings = 32%). Similarly, *F. neoclara* workers that performed the same task were more closely related than all workers that performed different tasks ($F_{1,292} = 95.31$, $p < 0.001$, Fig. 3.1B). According to analysis with COLONY, *F. neoclara* workers that performed the same task were siblings 77% of the time (full siblings = 52%, half siblings = 25%), while workers that performed different tasks were siblings only 40% of the time (full siblings = 26%, half siblings = 14%). In contrast, *F. podzolica* and *F. glacialis* workers that performed the same task were not more closely related than those that performed different tasks (Fig. S3.1). For all species, there was no association between matriline within polygyne colonies and worker size.
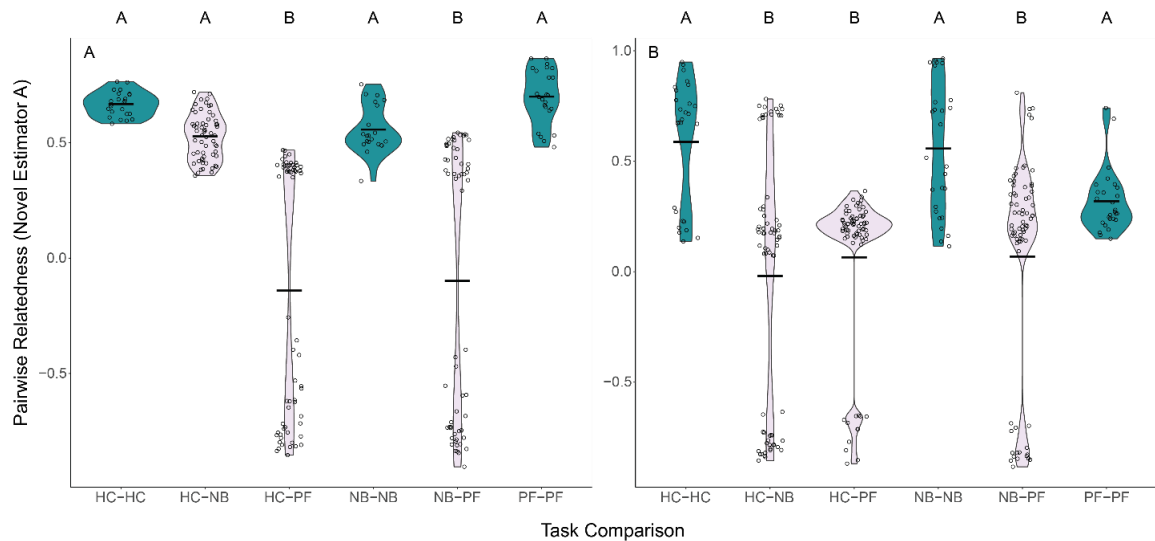
Figure 3.1: Violin plots depicting the pairwise relatedness of *F. obsuriventris* (A) and *F. neoclara* (B) workers, depending on whether they performed the same (blue) or a different (gray) task. Pairwise comparisons between all three tasks – honeydew collection (HC), nest building (NB), and protein foraging (PF) - are shown. Letters above each violin signify whether there is a significant difference in the average pairwise relatedness (shown with horizontal lines within each violin) of workers within each task comparison, based on a Tukey's range post hoc test.

Associations between Social Form, Worker Size, and Intracolony Variance

In *F. obscuriventris*, polygyne and monogyne colonies had similar average worker size (Fig. S3.2A). However, workers within polygyne *F. glacialis* ($F_{1,64} = 24.36$, $p < 0.001$), *F. podzolica* ($F_{1,63} = 14.48$, $p < 0.001$), and *F. neoclara* ($F_{1,67} = 12$, $p < 0.001$) colonies were significantly smaller than workers within monogyne colonies (Fig. S3.2B-D). For all species, monogyne and polygyne colonies had similar coefficients of variation.

Genome-Wide Association Study

In *F. obscuriventris*, several single-nucleotide polymorphisms (SNPs) were significantly associated with size and task (Fig. 3.2). In total, six SNPs were associated with worker size (Fig. 3.2A), 99 SNPs were associated with honeydew collection (Fig.

3.2B), and 32 SNPs were associated with protein foraging (Fig. 3.2C). Some significant associations were shared across phenotypes. Specifically, four SNPs were significantly associated with both size and honeydew collecting and two SNPs were significantly associated with both honeydew collection and protein foraging (Fig. 3.2). Three of the significantly associated SNPs are located on chromosome 3. There were no SNPs significantly associated with nest building (results not shown).
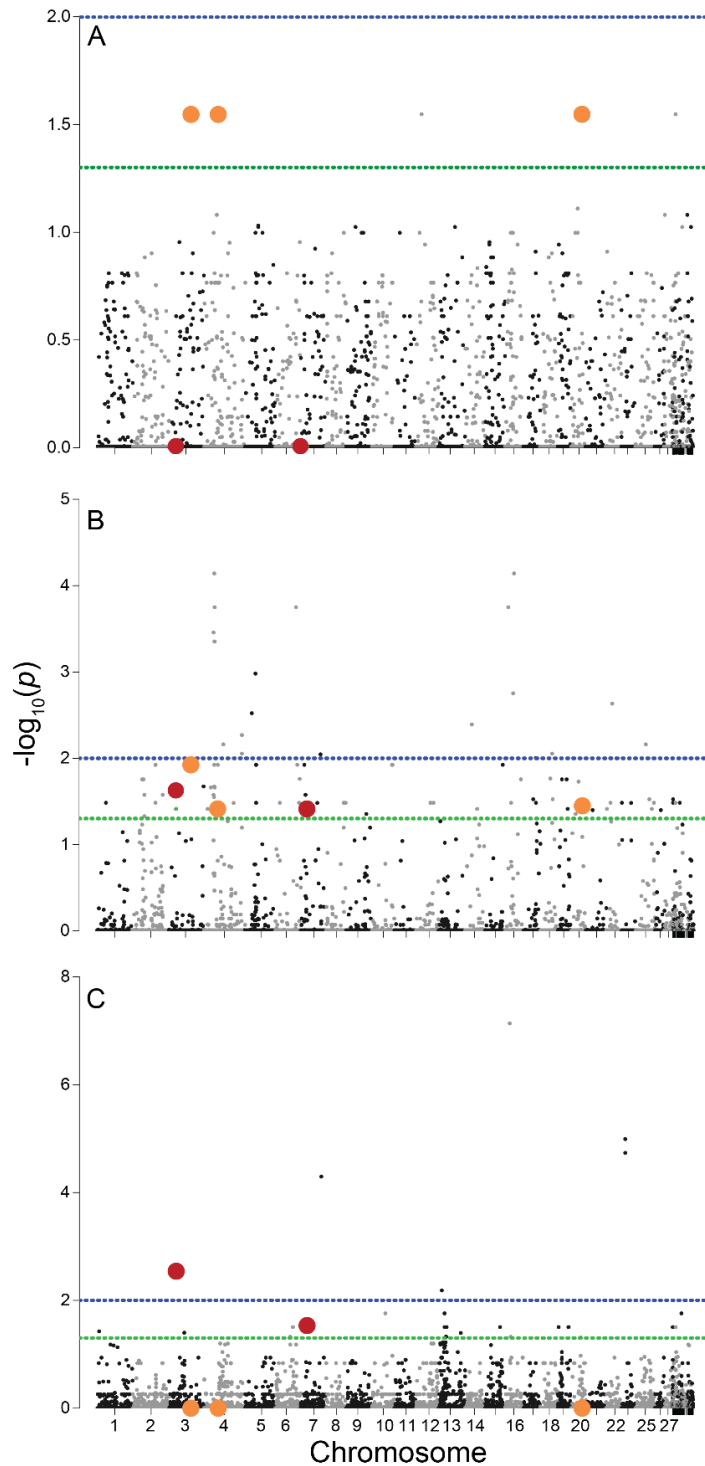
Figure 3.2: Manhattan plots showing the locations of SNPs significantly associated with worker size (A), honeydew collection (B), and protein foraging (C) in *F. obscuriventris*. p-values are corrected based on a false discovery rate. Green and blue dashed lines on each plot signify the threshold for p-values less than 0.05 and 0.01, respectively. SNPs significantly associated with both honeydew collection and size (orange) or protein foraging (red) are highlighted.

In *F. neoclara*, two SNPs were significantly associated with protein foraging, one each on chromosomes 4 and 9 (Fig. 3.3). There were no SNPs associated with worker size, honeydew collection, or nest building.
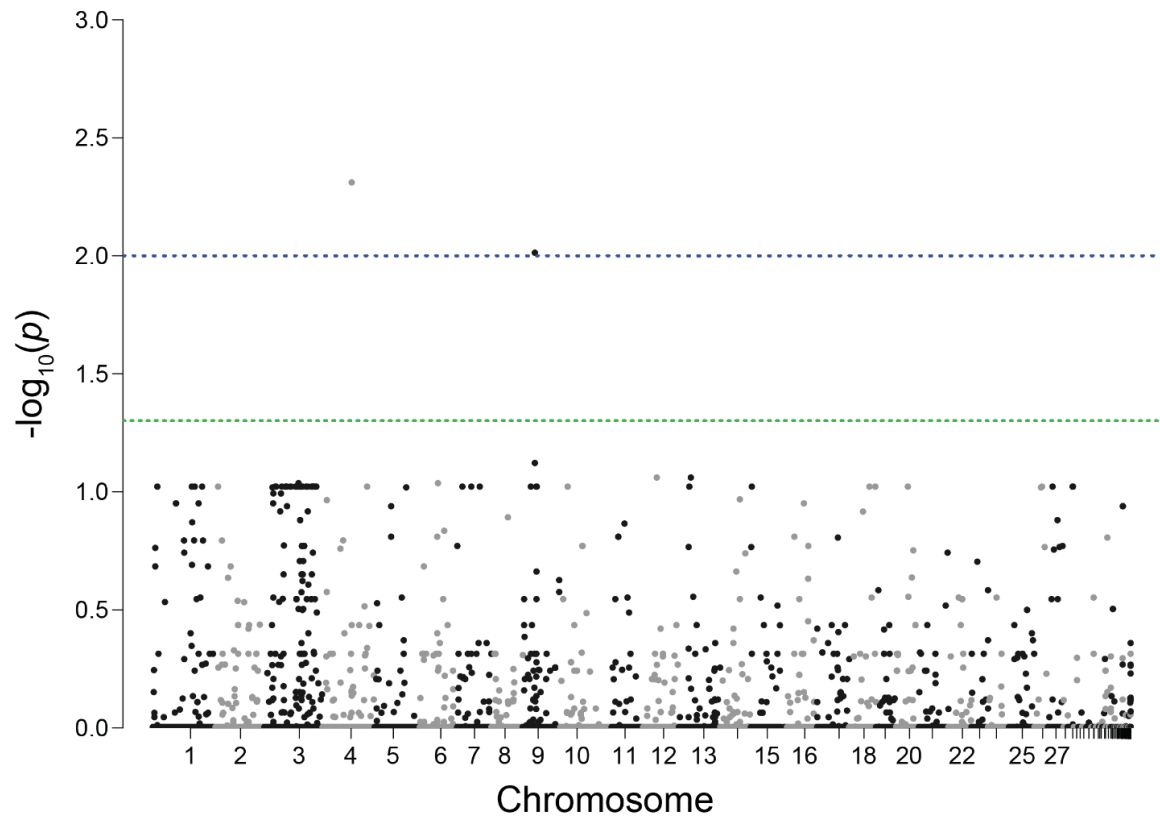
Figure 3.3: A Manhattan plot showing the location of SNPs significantly associated with protein foraging in *F. neoclara*. p-values are corrected based on a false discovery rate. Green and blue dashed lines signify the threshold for p-values less than 0.05 and 0.01, respectively.

In *F. glacialis*, 43 SNPs were significantly associated with worker size (Fig. 3.4A). All except one (located on chromosome 7) are located on chromosome 3. Similarly, in *F. podzolica*, 6 SNPs were significantly associated with worker size, three located on chromosome 3 and three on chromosome 4 (Fig. 3.4B). In both species, there were no SNPs significantly associated with task.
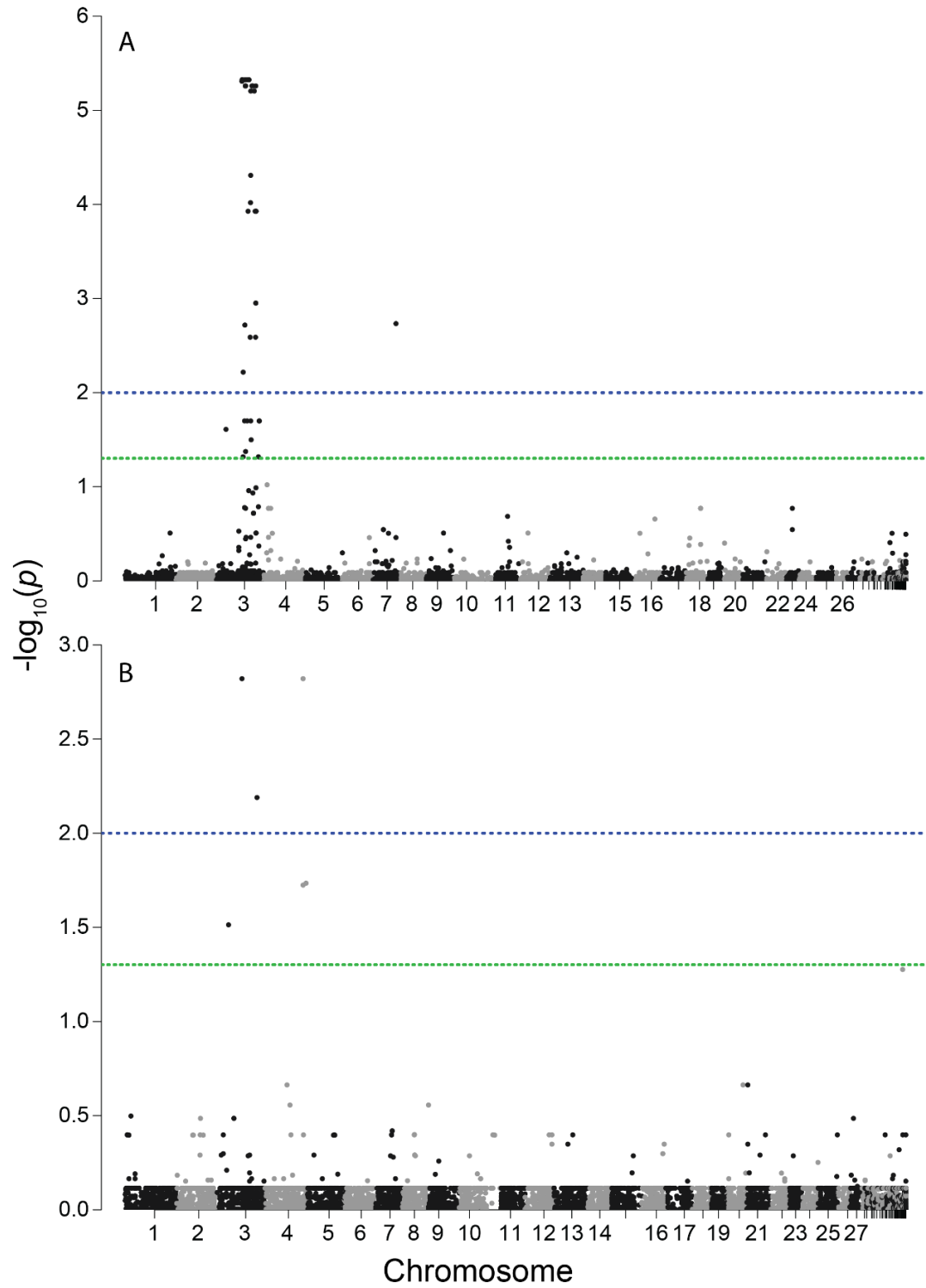
Figure 3.4: Manhattan plots showing the location of SNPs significantly associated with worker size in *F. glacialis* (A) and *F. podzolica* (B). p-values are corrected based on a false discovery rate. Green and blue dashed lines on each plot signify the threshold for p-values less than 0.05 and 0.01, respectively.

Discussion

In congeneric, socially polymorphic ant species, some species utilize a worker size-based task partitioning strategy. In this study, we show that task partitioning and size have a genetic basis in some of these species, but not in others. In two of five species, a worker's task is strongly associated with its matriline, while in two other species there is no association between genetic lineage and task. Matriline is not predictive of worker size in any of the four species, but in three species, we identified genetic regions that are significantly associated with worker size.

Of the five species we examined, *F. obscuriventris* has the most size variable workers and the strongest association between worker size and task (West & Purcell 2020). In this species, there is also a strong association between task and genetic lineage (Fig. 3.1A). Workers that perform the same task were siblings 97% of the time. Honeydew collectors and nest builders were also often siblings (77%), while protein foragers were more distantly related to workers that performed different tasks (siblings = 41%). There was no evidence of an association between genetic lineage and worker size in this species, and monogyne and polygyne colonies had workers of similar average size (Fig. S3.2A) and variance. However, our genome-wide association study suggests that worker size and task are non-independently associated with genetics, at least in some cases. We identified several genetic regions significantly associated with size, honeydew collection, and protein foraging (Fig. 3.2). Four SNPs are significantly associated with both worker size and honeydew collection, suggesting that these two phenotypes are genetically linked. It is intriguing that three of the SNPs significantly associated with worker size, task, or both

are located on chromosome 3, the 'social supergene' that controls whether a colony is monogyne or polygyne, as it suggests an association between social form, size, and task (Purcell et al. 2021). There is no previously known connection between social form and task in *Formica* and, for this species, we did not see a correlation between social form and size (Fig. S3.2A), unlike for the other species, in which polygyne workers are significantly smaller than monogyne workers.

In *F. neoclara*, we also see a strong association between genetic lineage and task (Fig. 3.1B). Workers in this species that performed the same task were siblings 77% of the time, while workers that performed different tasks were siblings only 40% of the time. Additionally, we identified two SNPs significantly associated with protein foraging on chromosomes 4 and 9 (Fig. 3.3). We did not detect any association between worker size and genetics in this species. Workers in polygyne colonies were significantly smaller than those in monogyne colonies (Fig. S3.2D), and we would expect monogyne and polygyne workers to differ genetically at the 'social supergene,' located on chromosome 3 (Purcell et al. 2021). Thus, it is interesting that we did not identify any size-associated SNPs along chromosome 3. However, our sample only included one monogyne colony, while the remaining five colonies were polygyne. Additionally, previous work suggests that the correlation between worker genotype and social form is not perfect in *F. neoclara*, with polygyne colonies often containing some workers homozygous for the monogyne-assocaited supergene haplotype (McGuire 2021). Thus, our GWAS for this species may not have had a sufficient sample of monogyne colonies to detect genetic

differences between monogyne and polygyne workers, especially since monogyne and polygyne workers do not always differ genetically.

In *F. glacialis* and *F. podzolica*, we do not find any evidence of genetic control of task. Genetic lineage was not associated with task, and we did not detect any SNPs significantly associated with task. Genetic lineage was also not associated with worker size. However, we did identify several SNPs significantly associated with worker size for both species (Fig. 3.4). The large majority of the size-associated SNPs in *F. glacialis* and half in *F. podzolica* are located on chromosome 3. Because these SNPs are located on the 'social supergene' and workers in polygyne *F. glacialis* and *F. podzolica* colonies are significantly smaller than those in monogyne colonies, it seems likely that these SNPs are indicative of differences in social form rather than task-related size differences.

Overall, this study suggests that both worker size and task may be subject to genetic control, but inconsistently across species. In only one of five species (*F. obscuriventris*) both phenotypes are genetically linked. In the remaining three species for which our sample included both monogyne and polygyne colonies, genetics only seems to play a role in either worker size or task, but not both. This is somewhat consistent with the findings of Schwander et al. (2005) in *F. selysi*, in which worker size and task were independently linked to genetic lineage. Unlike in *F. selysi*, genetic lineage was never predictive of worker size in any of the species we investigated. However, in at least two of the species (*F. glacialis* and *F. podzolica*), worker size does seem to be correlated with genotype along chromosome 3, which determines social form. Workers in polygyne colonies of these two species are also significantly smaller than those from monogyne

colonies (Fig. S3.2B-C), suggesting that the genetic underpinnings of size in these species has more to do with the life history than task partitioning. In the other two species (*F. obscuriventris* and *F. neoclara*) both matriline and genotype are associated with worker task. Previous work suggests that *Formica* workers exhibit task fidelity, at least over short periods (West & Purcell 2020). The association between task and genetics found in this study may suggest that task specialization occurs over longer periods than we previously had evidence for, at least in *F. obscuriventris* and *F. neoclara*.

It is noteworthy that worker size and task are genetically linked in only one species (*F. obscuriventris*) because all of the species utilize a size-based task partitioning strategy (West & Purcell 2020). However, behavioral observations suggest that the association between worker size and task is very strong in *F. obscuriventris* and much weaker in *F. glacialis*, *F. podzolica*, and *F. neoclara* (West & Purcell 2020). Although task proficiency has not been tested in *F. glacialis*, *F. podzolica*, or *F. neoclara*, our previous work suggests that specialization by large workers in nest building does lead to increased task efficiency in *F. obscuriventris* (Chapter II). Taken together, *F. obscuriventris* may both rely more heavily on and benefit more from a size-based task partitioning strategy than the other species we investigated. Thus, the shared genetic control of size and task in *F. obscuriventris* may be indicative of selection for and the ultimate fixation of size-based task partitioning in this species.

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Conclusion

Broadly, this research contributes to our understanding of morphological

polyethism in social insects that lack discrete morphological worker subcastes, an area of

research that has been largely neglected. Though many social insects possess continuous

worker size variation, non-reproductive division of labor based on body size has rarely

been examined. It is often assumed, on the basis of minimal empirical evidence, that

social insects without obvious morphological worker subcastes utilize temporal

polyethism. Even fewer studies have taken a holistic approach in considering the many

factors involved in this important aspect of social insect biology. This research, though

not entirely comprehensive, delves into some aspects of size-based task partitioning in a

widespread social insect genus, considering factors as diverse as task fidelity, behavioral

flexibility, efficiency, and genetics. Further, unlike many other studies that focus on a

single species, each chapter of this dissertation is comparative and ultimately examines how size-based task partitioning operates across multiple congeneric species.

In Chapter I, using a large-scale, multi-species field study, I show that *Formica* workers exhibit high task fidelity, and this task specialization is strongly associated with worker size. Specifically, large workers specialize in either nest building or protein foraging, while small workers specialize in honeydew collecting. This pattern was found across all the species that we tested, although the strength of the size-task association is strongest among the most size variable *Formica*. Several other social insects utilize a size-based task partitioning strategy (e.g., bumblebees: Richards 1946, Cumber 1949, Brian 1952, Jandt & Dornhaus 2009; sweat bees: Breed et al. 1978; wasps: O'Donnell & Jeanne 1995, Spradbery 1972) and many others possess size-variable workers, suggesting that it is a potentially widespread strategy. I argue that size- and caste-based task partitioning strategies are likely shaped by different selection pressures, are derived from different developmental processes, and have varying implications on behavioral flexibility (West & Purcell 2020). Thus, both deserve consideration when assessing the costs and benefits of morphological polyethism, because they may differentially contribute to how social insects fare under continually fluctuating environmental conditions, especially in the face of large-scale global change (Fisher et al. 2019).

In Chapter II, using a multi-species laboratory experiment, I show that, in two size-variable *Formica* species, there is a significant difference in task proficiency among workers of different sizes. Specifically, large workers, which specialize in nest building in nature, perform better than small workers at nest material collection in a controlled

laboratory setting, as predicted. In a third, less size-variable species, there is no difference

in the proficiency of differently sized workers at nest building. Contrary to our

predictions, however, small workers, which specialize in honeydew collection in nature,

did not outperform large workers at sugar-water collection. This may be due to

limitations in our experimental design to properly assess this task, or small workers may

provide advantages that we did not investigate. Further investigation is certainly needed,

but our results suggest that task specialization aligns with task proficiency in some, but

not necessarily all, cases. At the same time, *Formica* workers exhibit behavioral

flexibility. Laboratory experiments reveal that both large and small workers are capable

of performing tasks that they do not often perform in nature, albeit with varying levels of

success. In contrast, in species with discrete morphological worker subcastes, workers

that attempt tasks outside of their typical repertoire often perform them very poorly (e.g.,

Mertl & Traniello 2009). Thus, species that utilize a size-based task partitioning may fare

better than those that utilize a caste-based task partitioning strategy due to increased

behavioral flexibility among workers, particularly in times of stress or drastic

environmental change.

In Chapter III, in a multi-species genomic study, I show that *Formica* worker size

and task may both have a genetic basis, but this is species dependent. In two of five

species, workers that performed the same task were much more closely related than those

that performed different tasks, but matriline surprisingly had no effect on worker size in

any species. In the most size-variable species, both worker size and task are subject to

genetic control and may even share a genetic basis. In three less size-variable species,

either worker size or task were genetically controlled, but not both. Clearly, the genetic underpinnings of *Formica's* size-based task partitioning strategy vary across species, but the high genetic diversity found in polygyne colonies likely plays a significant role in some species.

Taken together, non-reproductive division of labor in *Formica*, like many other eusocial insects is highly complex. It appears that the most size-variable species do employ size-based task partitioning, and they may also reap the most benefits from this strategy in terms of task efficiency. In species with a high degree of intracolony size variation among workers, large workers both specialize in and perform better than small workers at nest building, suggesting that this strategy provides an adaptive benefit. However, in species with less variably sized workers, the association between size and task is weaker and worker size is not correlated with task proficiency. Further, the role of genetics in influencing size and task differs across species. Notably, worker size and task appear to be directly genetically linked in the most size variable species that we investigated, *F. obscuriventris*, but not in other species.

The differences in size-based task partitioning across *Formica* may suggest that the species within this genus are subject to different selection pressures. For example, differences in nest material and structure may lead to differences in the maximum possible size of workers, through limiting the size of nest tunnels and chambers. Indeed, our field study suggests that *Formica* species that build thatch-mound nests out of pine needles and wood chips generally possess workers of greater maximum size than those that build nests out of dirt and gravel, leading to differences in overall worker size

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variation across species (West & Purcell 2020). Naturally high intracolony worker size

variance in thatch-mound building *Formica* may have allowed these species to

experience greater benefits from size-based task partitioning, enabling selection to act on

and ultimately reinforce the use of this strategy. The fact that worker size and task share a

genetic basis in only the most size variable species that we investigated supports the idea

that size-based task partitioning has been strongly selected for in this species but not in

others. Ultimately, it is clear from this work that non-reproductive division of labor is

highly variable, even amongst congeneric species. It is my hope that this work sheds light

on this complexity and encourages other social insect biologists to challenge the notion

that a few broadly applied strategies are sufficient for describing the field as a whole.

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