**Behavioral underlining of hypometric metabolic scaling in the ant *Pogonomyrmex californicus***

**Conceptual Framework and Specific Aims**

Since the first half of the 20th century, we have known that larger animals burn fewer calories per gram of body weight than smaller animals (Kleiber, 1932; Kleiber, 1947), a phenomenon known as hypometric metabolic scaling (i.e., energy use scales sublinearly with body mass). This is a unifying pattern of biology observed from the scale of molecular processes up through the scale of whole ecosystems (Whiteside & Grzybowski, 2002; Kozlowski et al., 2003; Brown et al., 2004). As such, numerous hypotheses have been proposed to explain this phenomenon, ranging from delivery limitations of oxygen or nutrients (West et al., 2001) or because of decreasing demands for these resources (Glazier, 2010). Despite this intense study, this phenomenon’s proximate mechanisms as well as its ultimate evolutionary consequences remain controversial (Harrison, 2017; Glazier, 2018; Harrison et al., 2022).

A major barrier to studies of hypometric scaling is that it is difficult to manipulate body size without causing irreparable damage to the organism (Waters et al., 2017). However, hypometric scaling also occurs in social-insect colonies, where larger colonies burn less energy per gram than smaller colonies (Southwick, 1985; Jaffe & Hebling-Beraldo, 1993; Waters, 2014), and the number of individuals in a colony (i.e., colony size) can be directly manipulated while maintaining colony function. Furthermore, social insects are widely considered to be a superorganism (Holldobler & Wilson, 2009), as the group can be considered a coherent vehicle for gene propagation (Reeve & Holldobler, 2007). Therefore, the social-insect colony as “superorganism” is analogous to the organismic whole body, and understanding the principles that drive scaling in social-insect colonies may shield light on how this organismic phenomenon occurs more generally.

One hypothesis to explain the prevalence of hypometric metabolic scaling in social-insect colonies is that lower mass-specific metabolic rates of larger social insect groups reflect economies of scale generated from size-based changes in social organization, particularly in the organization of work. This benefit has been called “social synergy” (Jaffe, 2010) and is reflected in other animals (Peters, 1983; Creel & Creel, 1995). Thus, this hypothesis is driven by *reduced demands* at large sizes rather than more limited resource supplies. Task organization is generally divided into three components (Fewell & Harrison, 2016): division of labor (the degree to which individuals are specialized in a colony), task allocation (the relative distribution of work effort across tasks), and activity (the duration/intensity of task performance). Changes in these features could reduce mass-specific metabolic rate, which in turn could increase worker lifespan and reduce turnover of workers as the colony ages (Tschinkel, 1993; Tschinkel, 2006), and so they can be considered adaptive properties of larger colonies. Alternatively, supply limitations may explain hypometric scaling in colonies; internal organizational or energetic constraints may play more of a role in larger colonies (for instance, large colonies may suffer traffic congestion that can decrease movement) just as it has been suggested they analogously do in whole-bodied organisms (Brown et al., 2004).

For my dissertation, I propose to use harvester ant colonies (*Pogonomyrmex californicus*) as a model system for studying: (a) **how features of task organization scale with colony size,** and (b) **how scaling of task organization affects colony metabolism.** Toward this end, I will address 3 main questions in my proposed research:

1. **What is the best sampling strategy for estimating different facets of task organization?**

In an ideal world, every ant within a colony would be observed continuously for many hours so that different aspects of the group (division of labor, task allocation) and the individual (task switching rate, bout lengths) can be measured with high precision. However, manual tracking of behavior is time intensive, and so such an ideal is unrealistic. The purpose of this question is to develop an optimal sampling strategy across ants in a colony for estimating different features of task organization. This will be done by observing the behavior of 9 ants for 3 hours a piece and then evaluating the performance of different subsampling strategies (Chapter 1).

1. **How does task organization change across ontogeny, and what is its impact on metabolic rate?**

Division of labor, activity, and task allocation have all been shown to change in the directions thought to reduce colony-level metabolic rate (i.e., division of labor increases, activity per capita decreases, and work shifts from brood care) across different stages of development. However, it is not clear if these changes are directly linked to hypometric scaling. By measuring colony size, task organization, and metabolic rate simultaneously, I can determine whether changes in task organization can result in decreasing mass-specific metabolic rates (Chapter 2).

1. **How does task organization and metabolic rate change across experimentally manipulated colony sizes?**

Whereas task allocation and activity have both been shown to change across colony sizes, the relationship between colony size and division of labor is more uncertain. In two different studies of harvester ants, division of labor has been shown to increase with colony size (in an ontogenic study) or to not change at all (in a size-manipulation study). This latter effect could have been the result of limited acclimation time after the manipulation. I will perform a similar study but will use a larger acclimation period and simultaneously measure metabolic rate and other components of task organization in age-controlled colonies (Chapter 3).

I will address these questions in a laboratory setting, drawing from behavioral, physiological, and computational techniques. By measuring colony size, task organization, and metabolic rate simultaneously, this set of experiments will generate a more integrated framework of the behavioral underpinnings to hypometric scaling in social-insect colonies. More broadly, these experiments could help illuminate key principles of how metabolism scales across the animal kingdom (and potentially multicellular organisms in general).

**Background**

**Hypometric scaling in whole-bodied organisms**

A hypothetical isometric model of metabolic scaling is that the metabolic rate (*q*) of an animal increases proportionally with body mass (*M*); that is, there is a relationship *q* ∝ *M*α where scaling exponent α=1 (i.e., a linear relationship). However, interspecific metabolic measurements of animals consistently yield sublinear scaling exponents between 0.5 and 0.9 (Gould, 1966; Calder, 1996; Glazier, 2005; Isaac & Carbone, 2010; Hou et al., 2010; Waters, 2014; Glazier, 2022), a phenomenon called hypometric scaling of metabolism (Harrison, 2018), or sometimes negative allometry of metabolic rate (Glazier, 2018). The same pattern holds for intraspecific comparisons (LaBarbera, 1986; Reiss, 1989; Apol et al., 2008; Glazier, 2009; Bonner, 2011; Waters, 2014), although there are some rarer cases where hypermetric scaling (where α > 1) has occurred within a species (Killen et al., 2010).

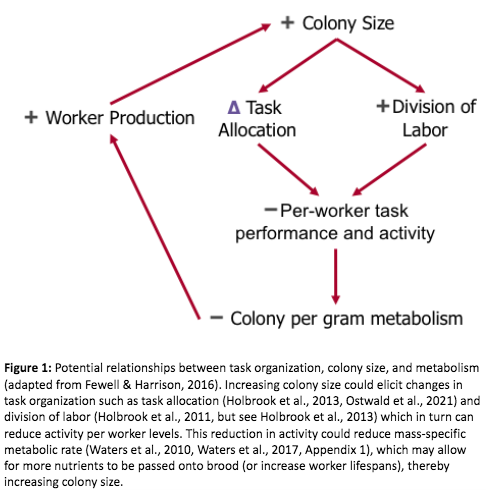
Numerous theoretical models posit that this allometric scaling is the result of physical constraints (i.e., supply-driven hypotheses). Some versions of the metabolic theory of ecology suggest that larger organisms are less efficient than smaller organisms because they have larger vascular networks, and thus have more nutrients in transit at any given moment. As a result, larger animals are incapable of utilizing those resources and thus burn less energy per unit volume (West et al., 1997; West, 1999; Brown et al., 2004; Maino et al., 2014; Brummer et al., 2017). Additionally, classical surface-area–volume models hypothesize that animals reduce their metabolism as they get larger to avoid heat exhaustion (Dodds et al., 2001; Agutter & Tuszynski, 2011). Although many of these models have been statistically validated, it can be challenging to test them in whole-bodied organisms. For instance, a version of a surface-area–volume model predicts that metabolic scaling is constrained by fluctuations in resources across cell surfaces which are not empirically tractable to observe directly in whole-bodied organisms (Finkel et al., 2004). These hypotheses have some support as recently excised cells also exhibit hypometric scaling (Porter & Brand, 1995); however, these types of cell-based hypotheses cannot explain why this effect diminishes with cells grown in culture (Glazier, 2015). Part of the issue is that it is difficult to manipulate components of energy usage (such as ion transport across membranes) in these model systems. In contrast, it is relatively easy to ascertain the processes causing energy changes in social-insect colonies, as foraging and activity can be recorded without extracting individuals. In a sense, individual and system components of the colony can be assessed *in vivo*.

**Hypometric scaling in social insect colonies**

Every major class of social insects exhibit hypometric metabolic scaling, including bees (Southwick, 1985), termites (Muradian et al., 1999), wasps (Hou et al., 2010), and ants (Waters, 2014). It occurs across age-matched colonies (Waters et al., 2010), size-manipulated colonies (Waters et al., 2017), and across developmental time (Guo et al., *in submission*). Intraspecific metabolic scaling exponents across social insects fall within 0.5 to 0.9 range for both laboratory-reared and field-collected colonies (reviewed in Fewell & Harrison, 2016) just as it does for unitary animals. Although there are some exceptions to this rule (for instance, rock ants scale isometrically, Dornhaus et al., 2009), generally social insects exhibit hypometric scaling.

In some special cases, mass-specific metabolic rates decrease as a result of constraints on heat exchange. In honey bees, hypometric scaling can be explained by reductions in surface-area–volume ratios in thermoregulatory clusters (Southwick, 1985). This ratio reduction increases insulation, and so individual bees in larger colonies do not need to expend as much energy to achieve the same temperature. Some polymorphic ants also demonstrate hypometric scaling as larger colonies produce larger workers, which themselves have lower mass-specific metabolic rates (Shik, 2010). However, hypometric scaling also occurs in monomorphic ants as well which do not cluster (Waters et al., 2017). How does scaling occur in these latter cases?

A clue to hypometric metabolic scaling in monomorphic ants may lie in the observation that hypometric scaling only occurs with intact, functional colonies. Queenless colonies, like aggregates of other non-social organisms, scale isometrically (Waters et al., 2010; Schoombie et al., 2013). This demonstrates that something about group living influences metabolic scaling. This societal change is likely captured by shifts in the collective organization of work activities in the colony (Figure 1; Fewell & Harrison, 2016). Larger colonies tend to exhibit higher levels of division of labor (Holbrook et al., 2011) and could have more elite workers (Pinter-Wollman et al., 2012) that can positively influence each individual’s ability to perform work. For instance, foragers in larger honey-bee colonies are able to more effectively gather resources than those from smaller colonies (Donaldson-Matasci et al., 2013), thus reducing average activity across nestmates. Larger colonies may also perform fewer tasks on a per-capita basis (Waters, 2014) in part due to reductions in resources needed to support the rapid growth of younger colonies, as in reductions in brood production (Cao & Dornhaus, 2012). As colonies with higher proportions of brood mass tend to have more active individuals (Guo et al., *in submission*), this reduction in brood care may reduce activity overall. While activity can increase with colony size (Schmid-Hempel, 1990), larger colonies tend to have slower-moving workers than smaller colonies (Waters et al., 2010) and have more inactive ants (Waters et al., 2017). Activity is a key component of whole-colony metabolic rate as active workers have a higher metabolic rate than inactive workers (Lighton et al., 2015). These reductions in activity can therefore result in decreasing metabolic rates, which in turn can increase worker lifespan (Tschinkel, 2006) and potentially lipid stores on the individual level (Tshinkel, 1999b). Worker production also increases or remains constant with increases of colony size (Waters et al., 2010). Therefore, these changes in task organization can contribute to the continued growth of the colony (Figure 1).



**Figure 1:** Potential relationships between task organization, colony size, and metabolism (adapted from Fewell & Harrison, 2016). Increasing colony size could elicit changes in task organization such as task allocation (Holbrook et al., 2013; Ostwald et al., 2021) and division of labor (Holbrook et al., 2011; but see Holbrook et al., 2013) which in turn can reduce activity per worker levels. This reduction in activity could reduce mass-specific metabolic rate (Waters et al., 2010; Waters et al., 2017; Guo et al., *in submission*), which may allow for more nutrients to be passed onto brood (or increase worker lifespans), thereby increasing colony size.

**Changes in task allocation during ontogeny**

Beyond shifts in brood production, colonies of different ages may perform a different set of tasks which could influence the metabolic rate of the colony. Life-history theory posits that animals at different life stages and body sizes will prioritize different fitness goals (Calder, 1996). For instance, small animals should invest heavily in neuro-locomotory performance so that they can outmaneuver larger animals (Stearns, 1992). Small animals have relatively larger brains than large animals (Prothero, 2015), and their skeletal muscles have higher mitochondrial content (Weibel & Hoppeler, 2005). Small animals, then, will prioritize performance over other considerations such as safety even if it means investing in more metabolically expensive tissue (Aiello & Wheeler, 1995; Harrison, 2017).

Similar adaptations can provide fitness benefits to young social-insect colonies as well. In social insects, faster growing colonies are more likely to survive inception and the ergonomic phase of development than slower growing colonies (Cole, 2009) until they achieve a critical mass, at which point they switch from a growth phase to a reproductive phase (Cole, 2000). This early rapid growth is characterized by isometric metabolic scaling and is followed by hypometric scaling later in ontogeny (Riisgärd, 1998; Glazier, 2005; Pearson et al., 2022; Guo et al., *in submission*). This shift in metabolism could be the result of changes in task allocation, which is the relative distribution of colony effort across different tasks (Fewell & Harrison, 2016). For instance, smaller colonies could invest more in risky foraging strategies to promote growth and then switch to safe in-nest tasks when the colony is larger (Kang & Theraulaz, 2016). Additionally, the proportion of time spent performing brood care diminishes with larger colony sizes (Holbrook et al., 2011; Ostwald et al., 2021). Simultaneously, the prevalence of maintenance tasks such as foraging tend to increase (Gordon, 1996; Holbrook et al., 2011; Ostwald et al., 2021), and the production of males also increases (Smith & Tschinkel, 2006). However, it should be noted that this change in task allocation is not a purely ontogenic effect as similar behavioral shifts occur when colony size is directly manipulated (Holbrook et al., 2013); thus, task allocation may flexibly respond to aspects of colony status related to its size.

**Changes in division of labor across colony sizes**

Unitary organisms benefit from a division of labor among soma cells, and this stratification could underlie hypometric metabolic scaling (Glazier, 2018). Division of labor is the degree to which individuals within a group specialize in one or more tasks (Gorlick et al., 2004). It can also be described as a stable pattern of variation among workers with respect to the tasks they perform (Beshers & Fewell, 2001). Division of labor tends to increase with colony size across many social insects (Jeanne, 1986; Thomas & Elgar, 2003; Holbrook et al., 2011; Amador-Vargas et al., 2015) as well as social spiders (Wright et al., 2019), but it can still provide fitness benefits for young, small colonies (Ulrich et al., 2018; Enzmann & Nonacs, 2021).

Increasing division of labor can reduce energy expenditure on both a colony level as well as an individual level. For instance, increasing division of labor can decrease colony-level queuing delays (Karsai & Wenzel, 1998). Specialist-leaning workers can be more efficient than more generalized workers, switch tasks less frequently, or decrease demands on communication to coordinate on tasks (Fewell et al., 2009; Jaffe, 2010; Dornhaus et al., 2012; Lin, 2021). Reducing task switching can be especially beneficial: task switching has been linked to temporal delays (Leighton et al., 2017), and the presence of task-switching costs can apply evolutionary pressure for division of labor (Goldsby et al., 2012; Lachaud, 2015). Task experience can also decrease the time necessary to complete a task (Trumbo & Robinson, 1997; Theralauz et al., 1998; but see Dornhaus, 2008) and can positively influence collective behaviors such as raft formation (Avril et al., 2016). Additionally, ants can specialize in inactivity (Charbonneau & Dornhaus, 2015). Even if the number of specialist inactive ants may not increase with colony size (Holbrook et al., 2011; Ruel et al., 2012; Charbonneau & Dornhaus, 2015; Feng et al., 2021), large numbers of inactive ants may be a result of less work in the colony than workers available to do it, a condition which should further reinforce division of labor (Jeanson et al., 2007). All in all, each of these traits can result in lower per-capita energy consumption and mirror the advantages of soma cell specialization in whole-bodied organisms (Holldobler & Wilson, 2009).

Whereas in polymorphic species, morphological castes are thought to play a significant role in determining division of labor (Wilson, 1978), among monomorphic species other intrinsic differences between individuals are thought to underlie specialization (Julian & Cahan, 1999) such as response thresholds (Beshers & Fewell, 2001; Weidenmüller, 2004; Lynch et al., *in prep*). Increasing genetic variation via polyandry and polygyny can increase colony size as a token of fitness (Baer & Schmid-Hempel, 1999; Wiernasz et al., 2004; Matilla & Seeley, 2007), as it might provide a system of genetically based task specialization through response thresholds (Oldroyd & Fewell, 2007). Genetic diversity affects division of labor (Julian & Fewell, 2004), and because larger colonies are more genetically diverse (Herrman et al., 2007), variation among individuals could help drive hypometric scaling.

Despite this, a direct manipulation of colony size did not elicit a change in division of labor in harvester ants (Holbrook et al., 2013). This could be evidence for the notion that division of labor may not be directly tied to colony size but may be indirectly related via other features of task organization. However, given the strong correlation between colony size and division of labor, this interpretation seems unlikely. An alternative explanation is that this lack of an observed effect was due to a short acclimation period (36 hours) after the size reduction, as normal division of labor may not have had time to recover after the disturbance.

**Social synergy and other benefits of cooperation**

The term “social synergy” is derived from the general hypothesis that the internal interactions of complex adaptive systems result in synergies that allow groups to efficiently utilize resources (Fewell, 1988; Jaffe & Hebling-Beraldo, 1993; Jaffe, 2010). Social synergy could increase with group size and could thereby underlie hypometric scaling if synergy increases efficiency. These efficiencies can arise through the minimization of task-switching costs by division of labor (Jeanson & Lachaud, 2015), but they can also arise through cooperation among individuals (Elgar, 1989; Kerhoas et al., 2014). For example, groups can transport objects more easily than individuals as they can lift the object off the ground and thus reduce friction (McCreery & Breed, 2014). Army ant teams performing collective transport can also take on unique postures when carrying long objects, increasing efficiency by diffusing rotational forces. Indeed, efficiency here scales hypermetrically, as teams can carry heavier objects than individuals carrying fragments of that same object (Franks, 1986). Traffic flow models of army ants also require many interactions among individuals (Couzin & Franks, 2003) and flow significantly increases with larger group sizes (Fourcassié et al., 2010), indicating that self-organized traffic rules are not very effective at small group sizes.

Species with larger colonies also tend to utilize large-scale communication techniques that allow them to mass-recruit to food sources whereas smaller colonies tend to use individual exploitation (Beckers et al., 1989; Donaldson-Matasci et al., 2013). Larger colonies may also be able to respond more effectively to changes in task demand through increased contact rates (Greene & Gordon, 2007; Holbrook et al., 2011; Holbrook et al., 2013). They could also have more specialized reserve workforces that can respond to specific fluctuations in task demand (Leitner & Dornhaus, 2019), and larger groups can also hunt more diverse prey (Silva & Jaffe, 2002). Larger house-hunting ant colonies can make faster, more accurate decisions by pooling information (Cronin, 2016), perhaps saving energy in the long term. For instance, groups of ants can outperform singular individuals in difficult perceptual tasks through the use of positive feedback, although these same processes make the group less accurate than the individual for easier tasks (Sasaki et al., 2013). Finally, the metabolisms of fire-ant rafts scales allometrically but only for short periods of time (Ko et al., 2022).

**Possible size-induced constraints on metabolic scaling**

Many of the most highly-cited papers on the allometric scaling of metabolism focus on how constraints imposed on large bodies prevent the organism from being able to utilize energy efficiently (West et al., 1999; Brown et al., 2004; Glazier, 2022), usually from the limitation of some critical resource (Harrison, 2017). There are two ways in which a social-insect colony may become energetically constrained as it becomes larger (Waters, 2014). It can become limited by the raw availability of a resource or how effectively that resource is distributed. Commonly studied resources include oxygen (Snelling et al., 2011), nest or foraging space (Meyer et al., 2006; Chism et al., 2019), and nutrients (Kaspari et al., 2009). However, these constraints do not necessarily cause hypometric scaling in social insects. For instance, it occurs even when colonies are fed ad-libitum (Waters et al., 2010, Cao & Dornhaus, 2013), suggesting that restrictions on nutrients might not affect scaling exponents. The sizes of nests and foraging arenas tend to be held constant in these experiments, and thus space might also be a limiting resource. However, local density of workers remains constant despite colony size (Waters et al., 2010; Holbrook et al., 2011), and so this seems unlikely. Finally, as these nests lie inside above-ground boxes, it is unlikely that they suffer from the hypoxic environments seen at field sites (Nielsen et al., 2003). As the superorganism is composed of individuals dispersed through space, they can largely bypass oxygen constraints anyway (i.e., a relatively large surface-area–volume ratio).

Still, the effects of resource constraints are well recorded. Colonies have been shown to consistently defend intake targets across colony sizes and for different combinations of macronutrient availability (Dussutour & Simpson, 2009; Smith et al., *in prep*), and so it can be assumed that deviations from this target would be detrimental, especially as differences in protein/carbohydrate ratios in larval diets can influence metabolic scaling in adults (Nicholls et al., 2021). Additionally, it has been shown that larger colonies have proportionally smaller trail lengths than smaller colonies (Bruce & Burd, 2012), and so foraging trail geometry could also act as a constraint. Variation in foraging behavior could be especially relevant for harvester ants as the metabolic gains from finding seeds can be 1,000 times greater than the metabolic cost of gathering the seed (Fewell, 1988). Finally, local density of workers can influence population-level metabolic rates, but results here are mixed. Increasing density has been shown to increase per-capita metabolic rates (Cao & Dornhaus, 2008), decrease them (DeLong & Hanson, 2009), or have no effect at all (Waters et al., 2010).

**Temporal sampling strategies used to estimate behavioral traits**

Testing hypotheses for how task organization scales with metabolism requires measuring at least two behavioral traits: the degree to which individuals are specialized on different tasks and the rate at which they switch between these tasks. These two features are best estimated with competing sampling strategies. The former can be best estimated with instantaneous sampling, which is the recording of an individual’s behavior during preselected moments in time (Altmann, 1974). It is also called scan sampling when multiple individuals are studied at each timepoint (Altmann, 1974) and is often used to minimize sampling bias (Mann, 1999). By sampling a little bit throughout a long period of time, observations avoid autocorrelative effects from previous instances and thus the proportion of time the ant spends on each task can be accurately estimated. Instantaneous sampling can capture the bout length of a behavior if the interval length is shorter than length of the bout (Lehner, 1992). However, bout length can be difficult to estimate without a preliminary dataset, and instantaneous/scan sampling misses momentary events or rare states (Lehner, 1992).

Conversely, bout length can be better estimated with continuous sampling, which is the continuous collection of all the behavior an animal does in a particular duration of time (Rose, 2000). Continuous sampling is considered the gold standard of behavioral sampling as it simultaneously measures frequencies, durations, and the times at which behaviors are performed (Martin & Bateson, 1993). However, continuous sampling requires a great deal of manual labor when analyzing multiple individuals or if relevant timescales are long, and so frequent instantaneous or scan sampling techniques are sometimes used instead to approximate a continuous sample (Tacha et al., 1985; Mitlöhner et al., 2001). It can also overestimate bout length and degree of specialization if bout length distributions have an abnormally heavy tail (Lynch et al., *in prep*). An intermediate sampling technique which could combine the benefits of both sampling regimes is lacking in the literature.

**Study species: *Pogonomyrmex californicus* as model system for studying hypometric scaling**

One challenge of testing hypometric-scaling hypotheses in whole-bodied organisms is that most studies rely on correlational data (Heinrich, 1981; Smith et al., 2009; White et al., 2011), which makes it difficult to disentangle causal relationships. Direct manipulation of body size would entail the removal of critical tissues which could cause unfounded harm to an organism (Waters et al., 2017). It is also difficult to add mass to an organism. Metabolic rate could be measured as a single organism ages and grows; however, here it is difficult to unravel the effects of size and ontogeny such as changes in locomotive rates (Ingram, 2000) or the progressive accumulation of injuries (Enquist & Leimar, 1990).

On the other hand, ants are a generally easier study system for studying the underpinnings of hypometric scaling relative to whole-bodied organisms. Although field colonies can grow to have several-million workers (Porter & Hawkins, 2001), this is still orders of magnitude less than the number of cells in a typical animal, making it easier to quantify the effects of individual units. Nests can be transparent, and thus many aspects of their collective behavior can be directly observed (Gordon, 2015; Charbonneau & Dornhaus, 2015). Finally, an empiricist can either add workers from one colony to another (Dornhaus & Franks, 2006) or remove workers (Holbrook et al., 2013; Waters et al., 2017). This is possible because workers are less specialized than cells and are capable of performing a wide array of non-reproductive tasks within a colony (Hölldobler & Wilson, 1990), although colonies usually exhibit intermediate levels of division of labor (Dornhaus et al., 2009; Holbrook et al. 2011).

The harvester ant *Pogonomyrmex californicus* is an ideal model system for several reasons. First, they exhibit hypometric scaling with scaling exponents in the same range of that as whole-bodied organisms (Waters et al., 2010; Waters et al., 2017), making results of this study comparable to the broader literature. They are also ecologically successful in the southwest (Cole, 1968), and so they are well adapted to the Arizona lab environment where the research will take place. Finally, their lab-reared colony sizes span multiple orders of magnitude (~10 to ~1,000), maximizing the probability of detecting size-related changes in colony-level measurements.

**Chapter 1: What is the best sampling strategy for accurately estimating variation in task specialization?**

Characterizing different features of task organization requires measuring the behavior of individual ants with high fidelity. However, traditional sampling methods such as instantaneous sampling (Altmann, 1974) are unable to capture many relevant features of behavior. For instance, instantaneous sampling may allow for determining the proportion of time an ant spends on each task, but it would say nothing about the rate or order at which tasks are performed, and it may miss rare or transient tasks. Continuous sampling could capture all of these features, but it can be extremely time-consuming, especially if the relevant timescale is over hours or days (Martin & Bateson, 1993). I therefore investigate optimal strategies for sub-sampling continuous data from social insects that minimize error associated with behavioral metrics that are independent of the time they are taken (such as proportion of time spent on each task) as well metrics which are dependent on the time they are taken (such as bout length).

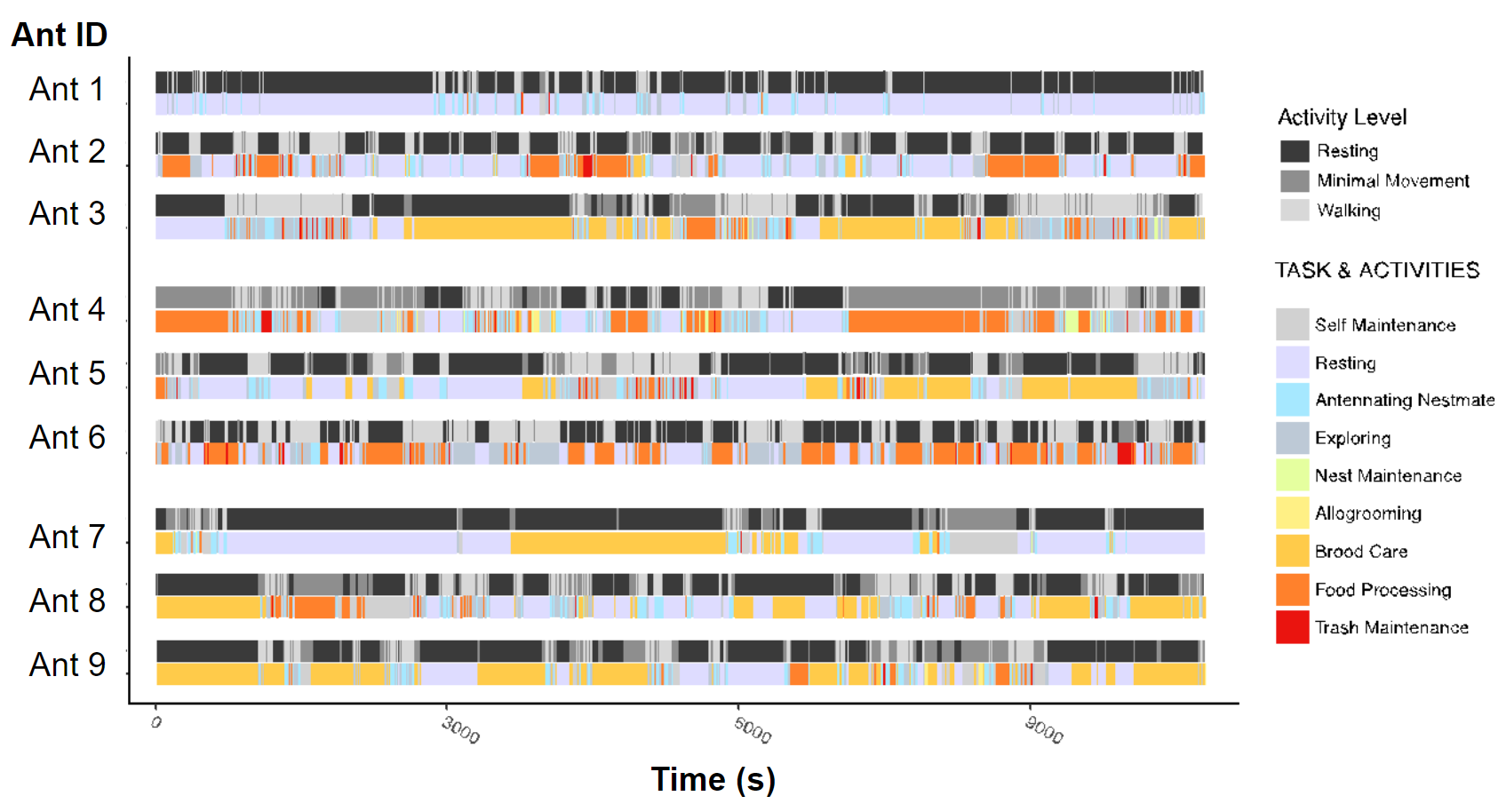
*1.1: Methods*

Dr. Bespalova collected and reared 2 *P. californicus* colonies in the lab (see GM 1.1. of General Methods section below). She recorded the behaviors of 3 ants from one colony and 6 ants from the other colony and categorized their behavior into 9 different states on a second-by-second basis for 3 hours (Fig. 2; Table 2). To estimate the hypothetical performance of different sampling techniques, we subsampled from this dataset. First, I found the sample size necessary to adequately reconstruct an ant’s fine-scale behavior. In particular, I found that sample size can be determined by finding a sampling scheme that would capture the rarest states once on average. I chose to design this sampling scheme around the rarest state as measurements of division of labor require good estimates for how work is distributed across tasks. If the rarest state is well estimated, then the more common states will likely have a good estimate as well. In this sampling scheme, it is assumed that the experimenter is randomly sampling instances from this dataset (where each instance represents a second) and determines whether the ant is performing the rarest task of the dataset; in other words, each sample from the dataset is a flip of a coin weighted by the probability that the sampled ant is performing the rare task. This sequence of Bernoulli trials results in a binomial distribution of the number of samples until the rare task is observed, and the expected value is the number of samples multiplied by the probability of observing the rare event during a sample. To ensure the rarest event is captured once on average over the sampling bout, we choose the number of samples by dividing 1 by the probability of encountering the rare event during a single sample. One ant had a probability of 0.0015 of allogrooming, and so to draw that state once on average we need to perform 660 trials. As each sampling trial corresponds to 1 second, this means that we need to observe each ant for 11 minutes each.

Next, instead of sampling single-second measurements at periodic intervals (instantaneous sampling; Altmann, 1974), I sampled continuous segments that were randomly distributed throughout the 3-hour span. I then varied the number of segments drawn from the dataset (each segment length is then the total sample size divided by the number of segments). I then measure how well differing numbers of segments can minimize two types of error. One type of error captured the difference between the proportion of time a sampled ant spent in each state to the true ant’s proportion of time in each state. The other type of error captured the difference between the average bout length of the sample versus the real ant. I finally determined which segment number minimized the sum of these errors, which were rescaled to ensure total error was not biased by one type of error or the other.

To ensure that these errors are not overly determined by these 9 ants, we also validated this process on a simulated dataset. The goal of these simulations is to produce behavior that is both similar and divergent from the real ants to see if different sampling strategies still work in these different contexts. The distribution of bout lengths often followed power laws rather than geometric distributions (Lynch et al., *in prep*), and so I opted to perform a discrete-event simulation to reproduce the behavior of these ants rather than a Markovian process. Prior to these simulations, I produced lists of bout lengths from the raw data for each individual state. During a simulation, I would randomly choose an initial state for the ant and then sample with replacement from the bout list that corresponds to that initial state. I would then randomly choose the next state (which had to be different from the initial state) and then draw from that bout list. I would repeat this process until the final behavioral vector is the same length as the original dataset (11,041 seconds). Across simulations, I adjust the probability of drawing from one state versus another to create a wide variety of differently behaving ants. These probabilities were drawn from a truncated geometric distribution whose range is between 1 and 9 (each value representing a state) and whose single parameter ‘*p*’ was varied such that probabilities of performing each task were uniform or extremely biased.

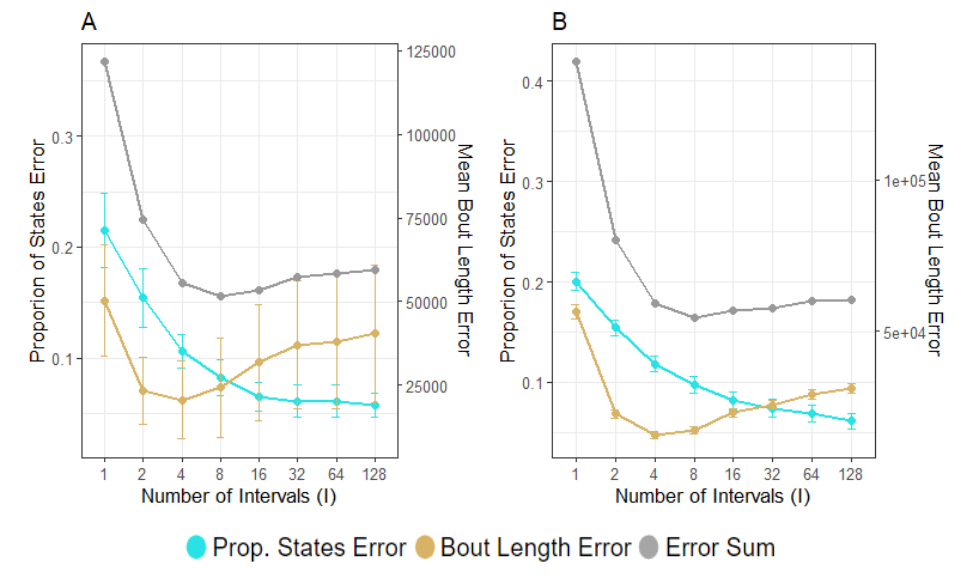
*1.2: Summary of Results and Discussion*



**Figure 2:** Visualization of the tasks each ant was performing every second. Each row represents an ant’s task performance through time. Each ant was categorized into either what task they were performing at a given second (color) or what their activity level was (grayscale).

Both types of error follow similar patterns in both the raw and simulated datasets. The error for bout length is U-shaped with its minima at the number of segments (*I*) = 4 (Fig. 3). Conversely, the error for the proportion of states continuously declines with increasing segmentation. This decrease in the proportion of states error moves the minimum of the sum of errors from *I* = 4 to *I* = 8.

While the exact minima is relevant for this dissertation as I will use this sampling strategy for Chapters 2 and 3, this result has some general applications as well. Simulations allowed us to control different aspects of the simulated ants’ behavior (such as the degree to which they are specialized on different tasks), and these differences in behavior had very little effect on the optimal outcome. I also performed this study on activity level with nearly identical results. All in all, it seems that sampling strategies where *I* ranges between 4 and 16 seem to measure different aspects of behavior better than either continuous sampling (*I* = 1) or scan sampling (approximated when *I* = 128).

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**Figure 3:** Independent and dependent errors across interval numbers for simulations and raw dataset. The left panel (A) shows ranges for the two types of error (probability of states error is teal, bout-length error is gold) across ants and/or simulations. Points show the mean error, and error bars show standard error. Note that these two errors are on different scales. (B) Sampling errors for simulated ants.

**Chapter 2: How does task organization change across ontogeny and what is its impact on metabolic rate?**

This experiment will determine how colonies change the regulation of their work throughout its development and what effect this may have on mass-specific metabolic rate. In reference to task allocation, I am interested in uncovering the relationships between brood, activity, and metabolic rate. Brood mass is proportional to activity, which itself is correlated with metabolic rate (Guo et al., *in submission*), but how is the presence of brood altering the behavior of adult workers? Previous studies indicate that brood care diminishes with larger colony sizes (Holbrook et al., 2013; Ostwald et al., 2021), as well as activity (Waters et al., 2010; Waters et al., 2017), although it doesn’t vary across colony sizes of age-matched colonies (Holbrook et al., 2011). Together, this suggests that the decline of brood care could be responsible for the decline in activity, which in turn can reduce metabolic rate. The presence of brood can influence other growth-related tasks such as foraging (Corneilius & Grace, 1997), which is a metabolically expensive task (Fewell, 1988). Therefore, reducing brood counts may also reduce foraging rates. Additionally, division of labor increases with age (Holbrook et al., 2011), and this could also reduce activity (Jeanson et al., 2007). Could changes in one or both of these aspects of task organization be responsible for hypometric scaling?

Here, Dr. Guo grew harvester ant colonies in the lab over several years (see GM 1.1 in General Methods section below), taking metabolic (GM 1.2) at the beginning and end of their development. I simultaneously monitored their behavior during these metabolic recordings (GM 1.3). Colony size is the independent variable that will first be linked to different features of individual and group level behavior such as task-switching rates and division of labor. These behavioral features will then be compared to whole-colony metabolic rate. In this chapter, size is confounded with ontogenic effects, and so it is unclear whether any given behavioral shift is due to changes over development or colony size. To control for this effect, Chapter 3 will examine behavioral shifts in size-manipulated colonies.

*2.1: Hypotheses*

While I will test many hypotheses in this study  including constraint-based hypotheses – I will primarily focus on testing the predictions of the task-allocation and division-of-labor hypotheses. Specifically, the predictions of these hypotheses in the context of three different research questions. Q1: How does each aspect of task organization change with colony size. Q2: How can changes in each aspect of task organization reduce activity. Q3: How does each aspect of task organization relate to mass-specific metabolic rate. Each hypothesis has a subhypothesis concerning each of these questions, and by testing each of these subhypothesis, I can potentially determine how changes in task organization reduce activity across colony sizes and thereby contribute to hypometric scaling (Fig. 1).

**Task Allocation H1:** Young/small colonies prioritize the growth tasks over maintenance tasks.

**Prediction:** Brood care/foraging is negatively associated with colony size. Other tasks are

positively associated with colony size.

**Division of Labor H1:** Older/larger colonies are able to exploit economies of scale and produce more specialized workers than smaller colonies.

**Prediction:** Division of labor is positively associated with colony size and negatively associated with mass-specific metabolic rate.

**Task Allocation H2:** Growth tasks are more strenuous than maintenance tasks, requiring more effort to complete.

**Prediction:** Ants performing brood care/foraging move more and therefore expend more

energy than they do when they perform other tasks.

**Division of Labor H2:** Specialist-leaning ants save energy by focusing on fewer tasks than generalist-leaning ants.

**Prediction:** Degree of specialization is negatively associated with task-switching rates and is positively associated with task completion.

**Task Allocation H3:** Growth tasks are more metabolically expensive than maintenance tasks.

**Prediction:** Energy spent on brood care/foraging is positively associated with

whole-colony metabolic rate. Other tasks are negatively associated or not associated

with mass-specific metabolic rate.

**Division of Labor H3:** A colony with high division of labor will have more specialist-leaning ants, which confer a number of energetic benefits to the colony, allowing the group to use less energy on a per-worker basis.

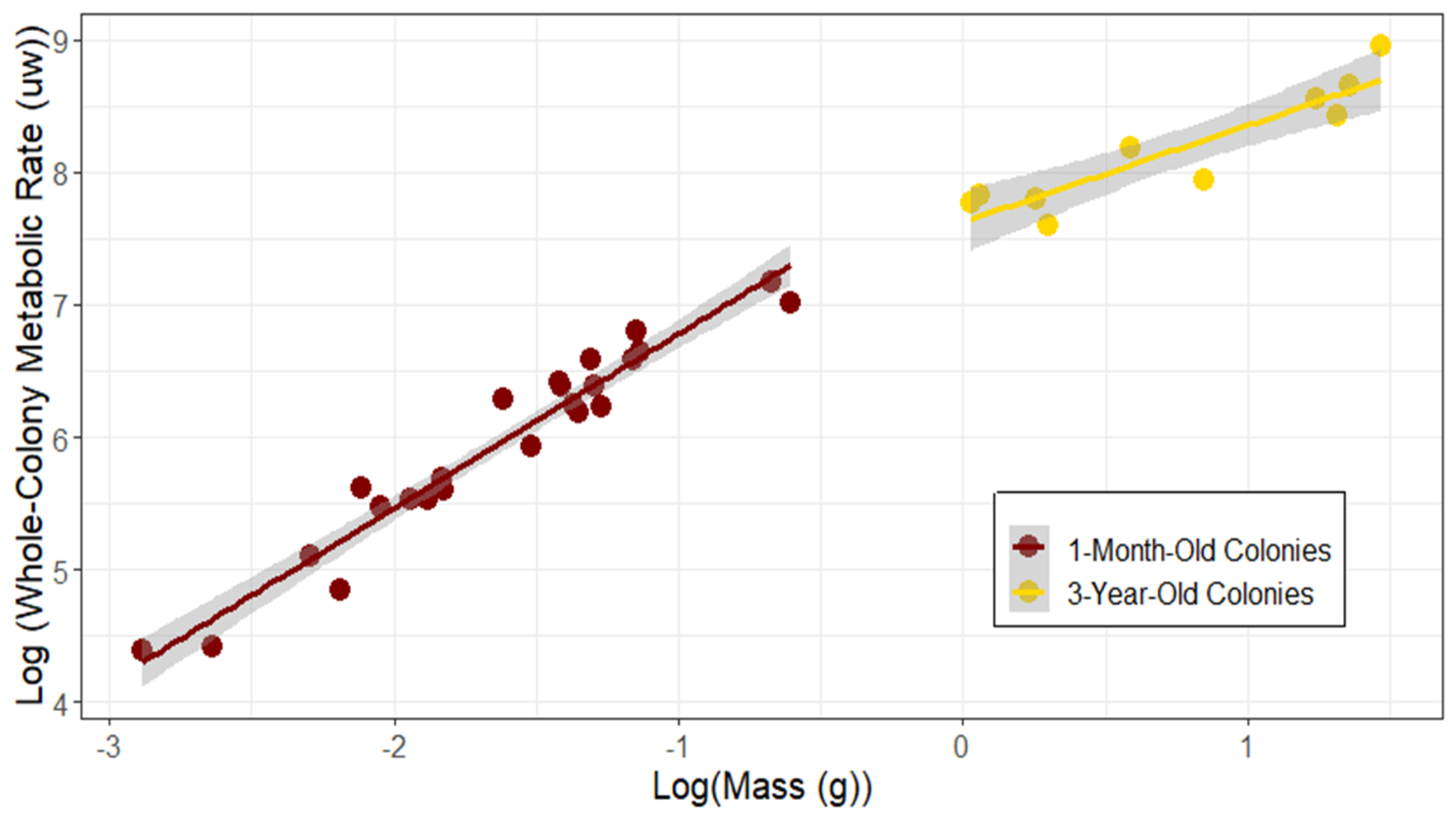
**Prediction:** Division of labor is negatively associated with whole-colony metabolic rate.

*2.2: Methods*

Dr. Guo raised 26 newly mated colonies in the lab (see GM 1.1 in General Methods section below), recording metabolism (GM 1.2) as well as behavior (GM 1.3) for 3 hours at two different stages of their developmental cycle (1 month vs 3 years). From the behavioral data, we estimate the amount of energy each ant spends performing each task based off of movement (GM 1.4). A separate experiment will be used to directly estimate the cost of brood care vs other tasks, as not all metabolic costs are linked to movement (GM 1.5). In addition, we count and weigh brood and workers each session. We have recorded the metabolism and weights of all colonies, but only 15 1-month-old colonies have been analyzed behaviorally so far.

*2.3: Current progress and summary of results*

The metabolism of young, small colonies scaled hypermetrically (Fig. 4, slope of linear model: 1.316, statistically greater than 1, *t* = –2.23, *p* < 0.05, 95% CI [1.183 1.449]) while the metabolism of larger, older colonies scaled hypometrically (Fig. 4, slope of linear model: 0.731, statistically less than 1, *t* = 4.48, *p* < 0.01; 95% CI [0.467 0.994]). This hypermetric scaling for young colonies could indicate energy may scale differently during early ontogeny for ants. In many mammalian species, allometric exponents are often higher than 0.75, and in some cases it is greater than 1 (Brody & Lardy, 1946; Holliday et al., 1967; Adolph, 1983; Hulbert & Else, 2005; Pearson et al., 2022). In these systems, this isometric and hypermetric scaling is attributed to the higher relative growth rates of internal organs that contribute disproportionately to resting metabolic rate (Hulbert, 2014). Similarly, hypermetric scaling in young harvester ant colonies could result from dynamic changes in internal structure that may not reflect task organization of a mature colony.



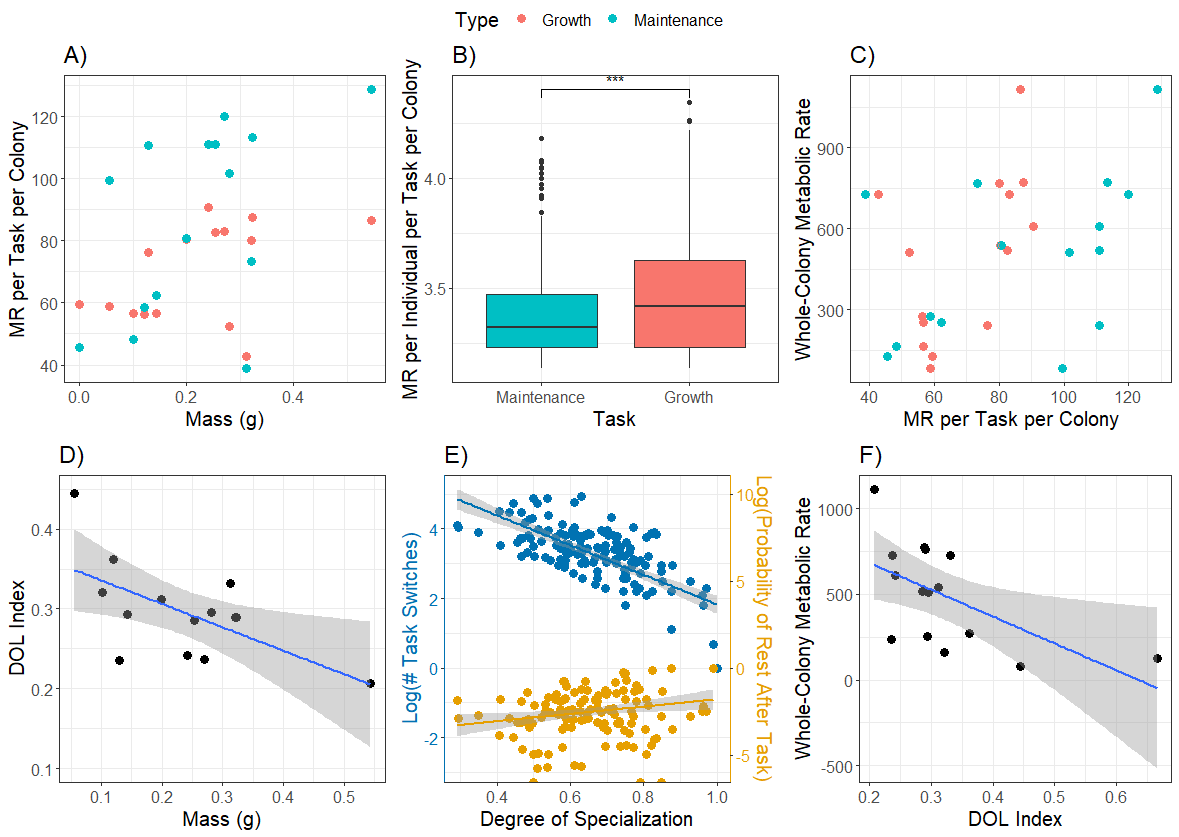
**Figure 4:** Hypometric scaling of young (red) and older (yellow) colonies. Regression lines display the results of a linear mixed model with colony ID as a random effect and age as a fixed effect (color). 1-month-old colonies have a slope of 1.316 (95% CI [1.183 1.449]) while 3-year-old colonies have a slope of 0.731 (95% CI [0.467 0.994]).

For instance, task allocation does not seem to change across colony sizes for 1-month-old colonies (Fig. 5A). To test task allocation H1, I categorized tasks as being either growth or maintenance tasks (Table 2) while excluding the rest state. I then summed metabolic rate contributions across individuals within colonies for each type of task (GM 1.4) and performed a multiple linear regression between as a predictor variable mass and movement energy expenditure as a response variable, with task type as a categorical factor. The model included an interaction between mass and task type. All factors in this model were insignificant (linear model mass effect *t* = 1.423,  *p* = 0.159; task type *t* = -1.098,  *p* = 0.282; mass/task type interaction *t* = -0.475,  *p* = 0.639). Additionally, I performed linear regressions between mass and the collective amount of time ants performed each task and found that there was no significant relationship between variables (linear model *p* > 0.05). The one exception is that the amount of time spent performing trash maintenance increases with colony size (linear model slope = 727.33, *p* < 0.05, *n* = 15). In short, we fail to reject the null hypothesis for task allocation H1.

To test task allocation H3, I performed a similar multiple linear regression with task type as a categorical factor, but here MR from movement is the predictor variable while whole-colony metabolic rate is the response variable (Fig. 5C). The model also included an interaction, but this time between MR and task type. Again, all factors of the model were insignificant (linear model mass effect *t* = 1.159,  *p* = 0.673; task type *t* = 0.25,  *p* = 0.804; mass/task type interaction *t* = 0.733,  *p* = 0.471), indicating that the amount of energy spent on each type of task did not correlate with overall metabolic rate. This is surprising, but it should be noted that only a small portion of individuals in a colony were sampled here, which when coupled the small sample size of colonies, means that we may need to increase power before we see a relationship. We therefore also fail to reject the null hypothesis for H3.

The significance of the main effect of task type, though, is evidence for task allocation H2 (Fig. 4B). When we compare the energy expenditure of each task to one another, we find that the 3 growth tasks each cost more movement energy than all of the maintenance tasks. Here, I found the amount of movement energy spent on each task for each individual and performed a Mann-Whitney-U test across task type(Fig. 5B; *W* = 69,778, *p* < 0.001). I found that growth task were on average more expensive than maintenance tasks, thereby supporting task allocation H2.

Conversely, changes in division of labor across colony sizes likely influenced metabolic rate (Figs 5D–F). In these young colonies, division of labor decreased with colony mass (linear model slope = –0.293, *t* = –2.698, *p* < 0.05). This may at first seem to contradict the prediction for division of labor H1; however, as these colonies are hypermetrically scaling, this is the expected result, as division of labor is still inversely proportional to whole colony metabolic rate (Fig. 5F; linear model slope = –1567.1, *t* = –2.616, *p* < 0.05). Colonies with high levels of division of labor have more specialist-leaning ants, which switch tasks less often and have a higher probability of resting after performing a task, a weak indication that they could be completing tasks at a higher rate (Fig. 5E). Here, both the probability of resting and the number of task switches are heteroskedastic, and so I log-transform these variables so that I can perform a linear regression on a homogenous variable (linear model for prob. stopping: slope = 2.094, *t* = 2.581, *p* < 0.05; linear model for task switching: slope = –4.272, *t* = –11.46, *p* < 0.001) Therefore, all 3 of the division of labor subhypotheses are supported.

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**Figure 5:** Tests for task allocation hypotheses H1–H3 (top row) and for the division of labor hypotheses H1–H3 (bottom row). For panels A, C, D, E, F, linear regressions were performed between the independent variable (x-axis) and the dependent variable (y-axis). Significant regressions are plotted. In the top row, all growth tasks are red while maintenance tasks are green. In B), stars indicate the significance of a Mann-Whitney-U test to compare the medians of the two groups. In E), there are two dependent variables that are on different scales (task switching count is in blue, the probability of resting after performing a task is in yellow; both variables are log-transformed). The left axis gives the scale for the number of task switches, and the right axis gives the probability of resting.

In summary, it seems that changes in division of labor may influence hypometric scaling more than changes in task allocation in young colonies, especially because the amount of time spent performing brood care did not differ significantly across colony sizes. However, these two hypotheses may not be totally independent of one another. Division of labor may have decreased in larger colonies as they were performing at least one task that smaller colonies did not have to contend with: trash maintenance. The inclusion of a new task could have spread out the effort of workers, decreasing the degree to which they were specialized and thus also decreasing division of labor. Therefore, for these small colonies, an increase in colony size could have resulted in a change of task allocation which in turn reduced division of labor, which in part could have reduced the whole-colony metabolic rate.

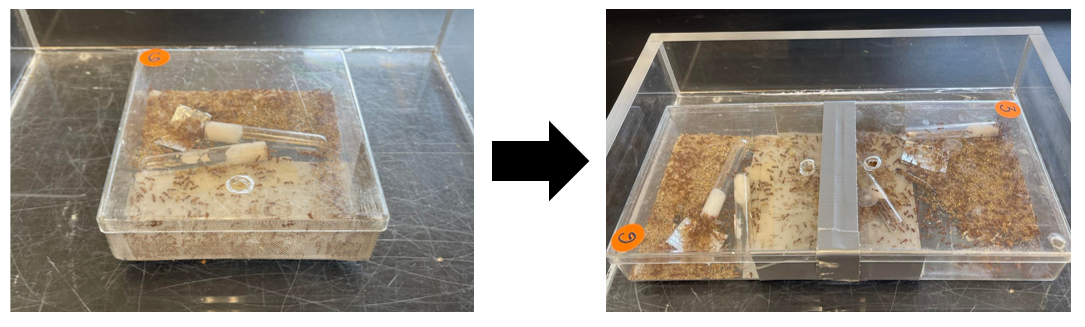
**Chapter 3: How does task organization and metabolic rate change across experimentally manipulated colony sizes?**

Division of labor increases with larger colonies in experiments where they are allowed to grow over time (Holbrook et al., 2011), but it does not change when colony sizes are directly manipulated (Holbrook et al., 2013). This could be an indication that division of labor shifts is an ontogenic effect, but this could also have been the result of the experimental design, where division of labor was measured 36 hours after the manipulation. Here, I will test whether division of labor will increase over longer acclimation periods and see how changes in division of labor may be associated with hypometric scaling. Changes in task allocation across ontogeny and colony size manipulation are more consistent but could use some clarification. Across the ontogeny of small colonies (between 1 and 10 workers), brood care decreased but walking and foraging time increased (Ostwald et al., 2021). Brood care also decreased among larger, size-manipulated colonies, but waste management and food processing increased instead (Holbrook et al., 2013). Finally, as colony size, metabolic rate, and task organization have to my knowledge not been measured simultaneously before, this experiment will also be the first to establish causal links between these phenomena.

This chapter will test the same hypotheses as Chapter 2 (see section 2.1), but it will control for developmental effects by creating a continuum of colony sizes through a process of fusing and then fissing colonies. Like with Chapter 2, metabolism and behavior will be measured simultaneously (see GM 1.1, 1.2, 1.3, and 1.4).

*3.1: Methods*

Ten colonies from Chapter 2 were randomly selected to fuse with one another in order to create 5 supercolonies (details listed in Fig. 6). This fusion was performed to increase the sizes of each colony and to blend the genetic material of each colony to help control for colony-level effects. Brood were lost during the fusion process, and so they are currently being nurtured in the lab until they return to normal brood numbers. After this acclimation process, colony sizes will be counted, and each of the 5 supercolonies will be divided into two smaller colonies. Colonies will be kept in the same sized nests, as increasing the local density of workers is unlikely to affect metabolic rates (Waters et al., 2010). However, changing the nest size could artificially change behavioral metrics I am tracking (for example, ants may seem faster in larger nests as they may contact fewer individuals on their route), and so I keep the nest size constant to avoid conflating colony size effects with nest size effects. Whereas queens will be evenly divided between each of the new colonies, workers will be divided in such a way that I have a wide range of final colony sizes. Brood will be divided proportionally according to the number of workers in each of the final colonies. After colonies have reacclimated to their new nests for 36 hours (Holbrook et al., 2013), I will take metabolic and behavioral recordings of each colony. I will take another measurement after 1 month, at which point the social structure of the colony should have stabilized. Similar to the previous experiment, I will measure colony size, metabolic rate, and behavioral measurements on the individual and colony levels.



**Figure 6:** All colonies were first placed in a sealed nest with wire mesh on one wall (left). To fuse this colony with another, two nests were placed together so that ants could anntenate and share cuticular hydrocarbons across the barrier (right). After 2 weeks, the ants, queens, and brood from both nests were placed into a new two-chambered nest where they were free to integrate.

Each supercolony currently has various numbers of workers (Table 1). Colonies will continue to be grown in the lab until the largest colony has over 1,000 workers. If populations plateau, then brood can be added from my spare colonies, or I can do another fusion with a healthy colony outside of this group. If I were to split the colonies today, I would divide them in such a way that the large colony resulting from the split would be *K* times larger than the small colony. This allows us to make pairwise comparisons between the large and small colonies while also creating enough variation in colony sizes that I can also use regression methods to test for nonlinear relationships between variables. If *N* is the current size, *N*S is the population of the small colony after fission, and *N*L is the population of the large colony, then *N* = *N*S + *N*L. *N*L = *KN*S, and so *N*S = *N*/(*K*+1) and *N*L = *N* – *N*S. By adjusting *K*, I can create a near uniform distribution of colony sizes. The exact value of *K* will change when final colony size is determined, but for now *K* = 6.05 works reasonably well (Table 1). However, the largest colonies after the split do not have over 1,000 workers, and so more work needs to be done to stimulate colony growth before fission can be attempted.

**Table 1.** N is the total colony size, NS is the size of the smaller colony after fission, and NL is the size of the larger colony after fission.

| **N** | **NS** | **NL** |
| --- | --- | --- |
| 823 | 117 | 706 |
| 565 | 80 | 485 |
| 729 | 103 | 626 |
| 250 | 35 | 215 |
| 435 | 62 | 373 |

**General Methods**

*GM 1.1: Ant collection and colony maintenance*

Newly mated *P. californicus* foundresses for all studies were collected on July 5, 2017, in Pine Valley, San Diego Co., California (lat 32°49′20″N, long 116°31′43″W, 1136 m elevation). Colonies are fed with ad libitum Kentucky bluegrass seeds, and provided frozen flies or mealworms weekly as well as bhaktar. Colonies are also given water via a test tube plugged with cotton and are maintained at a consistent temperature of 30℃. Colonies are kept in a nest partitioned into a foraging arena and a brood chamber, where the total surface area is 242 cm2. The exception is that the 1 month old colonies in Chapter 2 were in smaller nests with a surface area of 70.9 cm2.

*GM 1.2: Respirometry measurements*

CO2 emission rates from each colony are measured to estimate whole-colony metabolic rate using flow-through respirometry. Metabolic rate chambers are 0.74-L airtight aluminum respirometry chamber for 1 month old colonies from Chapter 2, and a 1-L airtight acrylic respiratory chamber is used for the 3 year old colonies and Chapter 3. The chamber lid is transparent to allow for video tracking of ant behavior. Dry, CO2-free air from a compressed air tank flows through the chamber (125 mL min-1 for the small chamber, 250 mL min-1 for the large one), regulated by Tylan mass flow valves and controller. An infrared CO2 & H2O analyzer (LI-7000, LI-COR, Lincoln, NE) is used to measure the carbon dioxide concentration of excurrent air. Excurrent air was passed through a Drierite column to remove water vapor as well as a Drierite/Ascarite column to scrub CO2. Room temperature and humidity levels are also measured simultaneously. Analog data is digitized (UI2, Sable Systems International (SSI), Las Vegas, NV) and recorded on a PC (ExpeData, ver. 1.2.6, SSI) at 1-Hz sampling frequency. Twelve-hours prior to CO2 measurement, colony enclosures are placed into the respirometry chamber with a loose lid for overnight acclimation. Seeds are placed into the chamber for nutrients, and several water tubes are placed in the chamber as well to prevent desiccation. During the daytime, the respirometry chamber is enclosed, and the CO2 from the whole colony is measured for approximately 3 hours after 1~2 hours’ washout. Measurements are made in a lab whose temperature ranged between 29 and 31° C. I assume respiratory quotient of 0.80 (Lighton & Bartholomew, 1988), and standardized the metabolic rate to the temperature of 25 °C to more easily compare our results to literature values, assuming a Q10 of 2.0.

For Chapter 2, carbon dioxide emissions were recorded over two sessions, one after colonies were placed in the lab (recordings were made between 9/17/2017 and 11/14/2017), and another recording was made well after the colonies had grown (recordings were made between 1/13/2021 and 2/5/2021). 26 colonies were measured in the first session, but only 11 survived through the second session. For Chapter 3, only one metabolic recording will be made after size manipulation is completed sometime this summer. Behavioral and respiratory recordings from Chapter 2 were done by Dr. Guo, whereas I will perform all measurements for Chapter 3.

*GM 1.3: Behavioral measurements*

While CO2 traces are being collected, a video camera captured the behaviors of all ants within both the nest and foraging chambers. In Chapter 2, session 1, all adult ants in a colony are marked with a 3 color code combination to identify individuals, but in session 2 only 20 randomly-selected ants are given a color code. This latter method will also be used in Chapter 3. Of these 20 ants, I manually record the behaviors of 10, choosing to paint more than necessary in case of die-offs.

Before observations were made, each video recording was spliced into 8 randomly-selected segments whose total length was 11 minutes (as determined in Chapter 1). For every second of this spliced video I recorded the spatial position of the ant (x-y coordinates are given in terms of the pixel count of the video, and I recorded whether they were in the nesting or foraging arena) as well as what task the ant was performing that second (Table 3). From this I derive colony-level metrics such as division of labor, task allocation, activity level, and so on. I also derive individual-level metrics such as velocity, degree of specialization, and the sinuosity of their paths (Table 4).

**Table 2.** Description of the 9 behaviors of individual ants.

| **Task** | **Description** | **Type** |
| --- | --- | --- |
| Brood Care | Carrying, manipulating, antennating, or resting on brood (larvae or live pupae) | Growth |
| Food Processing | Walking on food pile (intermittently antennating), carrying, manipulating, antennating, or resting on food (seeds, mealworms, or sacrificed pupa) | Growth |
| Trash Maintenance | Walking on trash pile (intermittently antennating), carrying, manipulating, antennating, or resting on trash. | Maintenance |
| Antennating Nestmate | Antennating Nestmate (worker or queen) while standing, or while walking if antennations happen within 10 seconds of each other | Maintenance |
| Nest Maintenance | Digging, excavating/biting nest, dirt, or cotton | Maintenance |
| Resting | Does not include resting on trash, food, or brood | Inactive |
| Self Maintenance | Self-grooming or being groomed | Maintenance |
| Exploring | Walking between tasks (not antennating nestmates for 10 sec), or trying to escape (climbing up the sides of the wall) | Growth |
| Allogrooming | Grooming nestmate (not being groomed by nestmate) | Maintenance |

**Table 3.** Description of metrics derived from raw spatial/behavioral data. The leftmost column determines whether a particular metric is at the individual or colony level, the second column gives the name of the metric, the third gives the definition of the metric, and the final column gives any relevant citations for that metric.

| **Level** | **Behavioral Metric** | **Definition** | **Citation** |
| --- | --- | --- | --- |
| Individual | # Unique Tasks | Count of how many different tasks an ant performed | NA |
| Individual | Degree of Specialization | Normalized sum of distances between how often an ant was performing each task and how often a 'perfect generalist' would perform those tasks. | Lynch et al., *in prep* |
| Individual | Prop. Time on Task | Proportion of time an ant spent on each task (Table 3) | NA |
| Individual | Bout Length per Task | Average length of time an ant spent performing each task (Table 3) |  |
| Individual | # Task Switches | Count of how often an ant switched between different states (# bouts – 1) | NA |
| Individual | # Antennations | Number of behavioral bouts which are antennations | NA |
| Individual | Movement Area | Area of the convex hull containing all points an ant visited. This was calculated separately for the nest and foraging arena. | NA |
| Individual | Sinuosity | Sinuosity of movement trail, calculated separately for the nest and foraging arena | McLean & Volponi, 2018 |
| Individual | Velocity | Average number of pixels transversed per second | NA |
| Individual | Interbout Interval | If an ant returns to a task after only being in one other state, then the interbout interval is the amount of time spent away from that task before returning to it | Leighton et al., 2017 |
| Colony | Division of labor indices | Degree to which individuals in a colony specialize | Gorelick et al., 2004 |
| Colony | Time spent on task | The total amount of time spent on a task across observed ants, calculated for each task (Table 3) | NA |
| Colony | Activity Proportion | Proportion of time all ants spent not resting | NA |
| Colony | # Coincidence tasks | The number of times the task (other than resting/self maintenance) is being performed at the same time by other ants. | NA |
| Colony | Coincidence task distance | If a task is being performed by two or more ants at the same time, then this is the average distance between those tasks | NA |
| Colony | Task Overlap | The area of intersection between the convex hulls of different tasks within the nest | NA |
| Colony | Brood Centrality | Euclidean distance between the centroid of the brood pile and the centroid of the nest | NA |
| Colony | # Lazy ants | Number of ants that rest for more than 50% of the time | NA |

*GM 1.4: Estimating energy from movement*

To estimate the amount of energy an ant expends from movement while performing different tasks, we measure their average speed while performing that task as well as how much time they spend performing that task. As we have an expression for the relationship between metabolic rate and movement speed (Weier et al., 1995), we can use this information to find the amount of energy used to perform these tasks:

*\text{MR} = \text{SMR} + \text{Y}_{el} + \text{MCOT}(\text{V}) 

*

where MR is the metabolic rate, SMR is the standard metabolic rate, and Yel is the intercept elevation above in J kg-1 s-1. V is speed and is in is in m s-1. Finally, the slope of the relationship between V and MR is MCOT, which is in J kg-1 m-1. To estimate the metabolic rate contribution of ant i performing task j from colony k *due to movement alone* (MRkij), we input the average speed of this ant performing this task (Vkij) into this equation. To my knowledge, SMR, Yel, and MCOT have not yet been measured in *Pogonomyrmex californicus*, however they have been measured in the closely related desert harvester ants *Pogonomyrmex maricopa* (Weier et al., 1995)*,* *Pogonomyrmex rugosus* (Lighton et al., 1993), and *Messor pergandei* (Lighton & Duncan, 2002). Our estimates for each of these parameters is therefore the mean across these 3 papers (SMR = 2.1 J kg-1 s-1, Yel = 1.03 J kg-1 s-1, MCOT = 137.97 J kg-1 m-1). While these estimates are unlikely to be exact replications of the real values of these parameters for *P. californicus*, they each constitute a linear transformation of the data, so the relative energy costs among tasks will be conserved regardless of which parameter values we choose.

*GM 1.5: Estimating energy cost of brood care vs other tasks*

Brood care is likely a metabolically expensive task whose performance is linked to hypometric scaling (Guo et al., *in submission*). Estimating energy costs of movement is almost certainly an underestimate of total costs, as work can still be performed at a single location. Even though *Pogonomyrmex barbatus* larvae likely perform the majority of digestion within the colony (as they excrete more fluid than brood care workers, C. Kwapich, personal communication), workers may also participate in digestion. Workers will squeeze/lick off the oil on the surface of seeds, and also perhaps could eat very fine grains of starch (McCook, 1879; I. Bespalova, personal communication). Additionally, particulate matter has been found in the stomachs of other harvester ant workers (Went et al., 1972), suggesting that workers are at the very least masticating, a task which may be energetically taxing while keeping the ant immobile (Garrett et al., 2016; Brown et al., 2017).

To estimate the metabolic costs of brood care relative to other tasks, I will employ a mask respirometric method. Mask respirometry is a pull-mode flow-through respirometry method where the respirometry chamber is built in the form of a mask that animals - typically mammals and birds - can breathe through freely. I can design a mask that is small enough to cover a brood pile within a nesting chamber as well as other parts of the nest where other tasks may be performed. This mask can be made of a transparent plastic, which will allow me to video record their behavior while we get metabolic readings so we can confirm which tasks are being performed within the mask. It will also give us a count of how many ants are present in the mask at any given time. The gaps in the mask will also allow ants to move in and out of it freely, minimizing the probability of an extended alarm response. I will push dry CO2 free air through the mask which will flush out atmospheric air. I will then suck air out excurrent air at about half the rate as incurrent air, allowing me to measure carbon CO2 emissions from workers and brood. I will first measure emissions at the brood pile while workers are tending their brood, and then I will make another measurement of the same patch of nest without workers, and one final measurement without brood. By subtracting the emission rates of these different sessions, I can estimate the metabolic rates of brood and workers separately. I will perform a similar experiment on another part of the nest where workers may be doing maintenance tasks. Here, though, only two measurements are necessary: one with and one without workers.

*Timeline*

a. Spring 2023

i. Perform colony fusion experiment

ii. Publish [temporal sampling](https://docs.google.com/document/d/1RrCbU3HX60hJ4d82mGaJNtazcmvMmzNWhj2PPZ5MP1o/edit), [colony sampling](https://docs.google.com/document/d/1_q44LVPWr1iMTpI9Lo_x01eZobVXeoQZNUejQHQdhq4/edit), [satisfaction threshold](https://docs.google.com/document/d/1mWxUlADxauZd97ZD6ZIQu_m_tITwkJzD0ZjOM24ioGI/edit), and

[generalist vs specialist](https://docs.google.com/document/d/1cf-x-h1G5ciATvHMZwj4NryorVYkGBRCyR9nq4Z2UEY/edit) papers

iii. Finish behavior video data collection for ontogeny experiment

b. Summer 2023

i. Measure metabolic costs of different tasks

ii. Publish [softmax threshold](https://docs.google.com/document/d/1YDyJpqyp0bd2U_u8E3waUWUrOfigI7Ie-BmKebXh9V4/edit) and [optimal threshold distribution](https://docs.google.com/document/d/1S2s3AQ6skAJgPfnOP7dkokDszDdPj1Vc23FCqCzHTH8/edit) papers

c. Fall 2023

i. Finish behavior video data collection for colony manipulation experiment

ii. Publish [casting machine learning](https://docs.google.com/document/d/1Easaozs1wXgo8kwrHAjqWTKjPPLs7cbbc_ilIQ3IF54/edit) paper on ensembles,

d. Spring 2024

i. Finish behavior video data collection for colony manipulation experiment

ii. Publish [Easter Island evolution of cooperation](https://docs.google.com/document/d/1K2TM3xnNYYKkTn0zitxl-BkoFgo8DBf1Q7bmiVy1QZg/edit) paper

iii. Finish behavior video data collection for task energy cost experiment

d. Fall 2024

i. Publish paper on [maximizing power of experimental designs](https://docs.google.com/document/d/1sFO7ki8JW8GtIIdPegIwbmYi3yc4m9tVVN91bsIHcCI/edit), [colony health](https://docs.google.com/document/d/1ZQHhbxv-gjWpHEc7Ltohe4xvNFdGM6-RqPn0qG7lsxU/edit)

[robustness to environmental perturbations](https://docs.google.com/document/d/1ZQHhbxv-gjWpHEc7Ltohe4xvNFdGM6-RqPn0qG7lsxU/edit) paper

ii. First drafts of final dissertation

e. Spring 2025

i. Finish writing final manuscript

ii. Publish ontogeny and colony size manipulation studies

f. Summer 2025

i. Defend dissertation

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