ECOLOGY AND EVOLUTION OF OFFSPRING SIZE IN *POGONOMYRMEX* COLONY FOUNDING

A Dissertation Presented to

the Faculty of the Department of Biology and Biochemistry

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In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

By

Lara Appleby

May 2013

ECOLOGY AND EVOLUTION OF OFFSPRING SIZE IN *POGONOMYRMEX* COLONY FOUNDING

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ABSTRACT

Mortality scales with individual size in many organisms. In social insect colonies, mortality peaks early in colony development, when colonies are young and small. The workers that compose these new colonies are extremely small individuals, called nanitics. I assume a size-based mortality schedule for social insect colonies and investigate the extent to which production of nanitics and other aspects of early colony development could be adaptations to the aforementioned mortality schedule. I ask specifically (1) whether temperature, food availability, and the social environment limit colonies in the production of their first brood of workers, (2) whether the size and number of workers in a colony's first brood affect the colony's growth rate, (3) whether adult worker size affects the production of a batch of new workers, and (4) whether the number of workers in the colony's first brood affect a colony's probability of victory in direct competition with another new colony. The absence of adult workers in a new colony limits the size and number of workers that the colony can produce. Colony growth in the first 8 weeks beyond the first clutch is limited by the size of the first clutch; colonies with larger first clutches grow more quickly. Adult nanitics may produce new workers as quickly as do adult non-nanitics. First clutch size does not affect the probability of colony survival in direct competition with other new colonies in the lab. Together these results suggest that early colony development has been shaped by selection for fast colony growth. Production of nanitics (1) may enable production of relatively large first clutches, which may accelerate colony growth and (2) seems to confer little if any cost in brood care. That colonies normally stop producing nanitics after colony founding could simply reflect an increase in the availability of resources for worker production, such that both the size and number of workers produced in a clutch increase with the same overall allocation pattern among worker size and clutch size as in new colonies. It is also possible that selection favors increased investment in worker size in later stages of colony life.

CONTENTS

1 Introduction

1.1 Context: Evolution and ecology of non-adult forms

TACTICS OF GROWTH AND REPRODUCTION

An organism's life history is the way the organism deals with the basic challenge of life: reproduction. Almost by definition, life history traits (e.g. body size at first reproduction, growth schedule, clutch size, age-dependent mortality rates) should impact fitness. Many life history traits must be considered in the context of each other, as reproductive tactics (Stearns 1976) because (1) traits vary in their effects on fitness and (2) the value of one trait can affect the value of another. To understand extant biological variation in life histories, we need to know not only how individual traits affect fitness, but also the extent and direction of interaction (e.g. synergism, constraint) among traits. The organism's environmental context (e.g. resource availability, predator abundance) can affect the relationship between trait values and fitness as well as the relationship among traits. For example, a trait that confers great resistance to predation has little effect on the dynamics of a population during a period of overall low predation (Sogard 1997). I use this integrative life history framework in my study of the dynamics of social insect colony founding.

Larval and juvenile life stages and growth patterns *per se* may, like adult traits, be subject to selection. In colonial marine invertebrates, juvenile growth rate during the larval stage corresponds to increased survival of the first few weeks of the juvenile stage, suggesting a size-dependent mortality (Marshall et

al. 2003, Marshall et al. 2005). In plants, seedlings that are initially larger are more likely to grow faster, but some of these studies were interspecific (Stanton 1984, George and Bazzaz 1999, Dalling et al. 2002). The high juvenile mortality present in many systems creates challenges for study in the field; because of the high mortality, studies must begin with extraordinarily large numbers of individuals. So the fitness consequences of traits and interactions among traits in non-adult forms are unknown in many systems.

SOCIAL INSECT COLONY GROWTH

Growth of a social insect colony is different from growth of a solitary organism, though it occurs in a similar phase of the life cycle. When solitary organisms reproduce, they make new individuals, which grow and finally reach reproductive maturity themselves. The growth phase consists largely of somatic cell growth and proliferation. When social insect colonies reproduce, they make reproductive individuals, which mate and start new colonies. The new colonies then grow before reaching reproductive maturity themselves and may continue to grow once they are reproductively mature. Colony growth consists largely of production of new workers with eggs made by the queen, and nutrition and care provided by the existing adult workers. The growth of a colony, then, involves production of nearly independent biological units (workers), arguably far less integrated and interdependent than the cells of a growing solitary organism. The somatic anatomy and physiology of workers and the reproductive anatomy and physiology of queens are nearly identical to that of solitary insects. This means

that classic somatic consequences of body size in solitary insects (e.g. desiccation resistance, metabolic rate) could apply in social insects to worker (and queen) size and the classic reproductive consequences of body size in solitary insects (e.g. egg-laying rate, egg size) could apply to social insect queens. These solitary insect-like consequences of body size may impact the ways in which social insect colonies grow, i.e. the rate of production of new workers and their sizes.

Throughout this dissertation, when I refer to colony growth, I mean a colony's increase in the number of adult workers that it contains. I also use some other terminology specific to social insects and especially social insect colony founding (defined in Table 1).

Table 1: Stages of colony founding

TERM	DEFINITION
alate	*fertile, winged male or female departing the parent nest or at the mating swarm*
foundress	*alate digging a (new) nest*
incipient colony	*queen and her first clutch*
juvenile colony	*an incipient colony that survived the winter, not yet reproductive/mature*

1.2 Background: The worker size distribution

COMMON DYNAMICS

Social insects in the order Hymenoptera are holometabolous which means that once an individual ecloses as an adult, its physical form or overall body size is fixed. A colony is composed of individuals of a variety of sizes. In the simplest case, a colony consists of a queen and workers. The main caste distinction in

social insect colonies is between worker and queen and that distinction is determined in large part by individual larval nutrition (Wheeler 1994); in other words the same genotype can develop into a worker or a queen. There is also body size variation within the worker caste. Some of this variation is over time, if the colony varies the size of worker it produces. Worker size can also vary within a colony at a single time point. The spread of worker sizes in a colony is called the worker size distribution. Some species have discretely polymorphic workers (morphological castes), that is, they have some workers of one size and shape and other workers of another size and shape (e.g. *Atta*, *Pheidole*). Some species have continuously polymorphic workers, that is, workers which span a range of sizes and have common shape, or allometry (e.g. *Pogonomyrmex barbatus*).

Worker size and variation in worker size increases with colony size in both kinds of species. The first workers made are extremely small, and are called nanitics. After colony initiation, *Solenopsis invicta* (fire ant) colonies produce minor and major workers, and an increasing proportion of a colony's workers are majors as the colony grows (Tschinkel 1988). This leads to an increase in mean worker size with colony size (Wood and Tschinkel 1981). After producing its first clutch (of nanitics), *Pogonomyrmex badius*, the Florida harvester ant, also makes both major and minor workers. In *S. invicta*, the first minor workers produced are very small, and the fat and lean weight of minor workers increases with colony size (Tschinkel 1998). Mean worker head width and variation in worker headwidth also increase in *Myrmicaria opaciventris* and *Myrmica rubra* colonies as they age (Brian 1957, Kenne et al. 2000).

This dissertation is a study of the causes and consequences of the small size of workers of a colony's first clutch. Walter Tschinkel (1998) explains the general goal of such a study:

> *The challenge of a life-history approach to social insect evolution is to identify which features are epiphenomena of no evolutionary importance, which are life history tactics and how these tactics affect colony reproductive success.*

That the size distribution of workers changes with colony size has been known for more than a century (Wheeler 1910), but the causes of worker size change remain uncertain.

My dissertation focuses on the worker size distribution during early colony life. In all independently-founded species, colonies begin growing by making small workers. These workers have been observed in a variety of species (summarized in Wood and Tschinkel 1981), but their causes and consequences have not been thoroughly examined. My dissertation is centered on the question: "Why don't new colonies make larger workers?" I want to know about proximate causes of new colony production of nanitics and the consequences of nanitic production for colony performance.

PROXIMATE CAUSES

It is likely that proximate factors contribute to the small size of nanitics and to the other colony-developmental dynamics of worker size and clutch size. Details of the environment's effects on body size and clutch size are well-understood in solitary insects (Fox and Czesak 2000) and similar factors have been found to

affect the workers produced by social insect colonies (summarized below). Temperature is one of these factors. At high temperatures, solitary insects produce larger clutches and the worker populations of social insect colonies grow at high rates (e.g. Porter 1988). However, in social insects, worker size does not respond to temperature the way solitary insect size does. Many solitary insects develop into smaller adults at higher temperatures (e.g. Davidowitz et al. 2004), but some are larger as adults when reared at higher temperatures (e.g. Honek 1992). In social insect colonies, offspring size can be regulated by the caretakers, or the adult siblings that provision the brood. For example, *S. invica* colonies produce workers of constant size, regardless of temperature fluctuation (Cassill and Tschinkel 2000).

Food availability is another environmental factor affecting insect reproduction (including offspring size and clutch size). Food abundance results in large adults in many solitary insect species and some social insects. The first workers made by *Pogonomyrmex californicus* colonies were larger when seeds were provided than when the queens were forced to rear the brood on internal reserves alone (Johnson 2004).

Social insect larvae can be affected not only by the abiotic factors of food and temperature, but also by the social environment. Insect larvae develop based on their rate of feeding and in insects with parental care this in turn depends on the rate of provisioning by the caretakers. In social insects, the brood is usually reared by adults in the colony, so larger colonies often make larger clutches and larger offspring. In *Solenopsis invicta*, for example, artificially larger

colonies produce larger pupae (Porter et al. 1985) and the first workers made by new queens in artificially large lab colonies were larger than nanitics (Wood and Tschinkel 1981). Likewise, smaller workers were produced by artificially small *Atta cephalotes* colonies (Wilson 1983). And in *Pogonomyrmex barbatus/rugosus* hybrid lineages, when the colony's offspring were queens, artificially larger colonies produce larger queens (Schwander et al. 2008). Care also affects colony growth rate in *Pogonomyrmex barbatus/rugosus* hybrid lineages, with artificially larger colonies growing faster in number of workers and producing more queens (Schwander et al. 2008). In colonies with multiple queens, queen number can also affect worker number and colony growth rate, but the extent to which this is via increased larval care (because of increased number of adults/caretakers), increased food availability (via trophic eggs laid by the queens), and/or increased per-capita egg-laying rate is unclear and variable among species (Kenne et al. 2000).

Though colony size often correlates with colony age in nature, these two factors may affect worker production independently. For example, new queens in artificially large *Solenopsis invicta* colonies produced non-nanitic workers (Wood and Tschinkel 1981).

Variation in not just quantity, but also quality of larval care within colonies across colony development may also contribute to worker size variation. One potential source of brood care quality variation is in the size of the brood care workers. *Solenopsis invicta* colonies composed of larger workers produce smaller pupae (Porter et al. 1985). Because those large-worker colonies were

composed of all majors, a subcaste of *S. invicta* that probably never comprises an entire colony in nature, these data do not constitute much support for effects of caretaker size on new worker size.

The proximate environment of larvae in new colonies has not been measured. It may be that temperature is high and labile. Colonies of many species are founded during warm parts of the year. Mature colonies can be quite deep and have external structures like mounds, and these different parts of the nest can vary in temperature (Cole 1994). Mature colonies of some species control brood temperature by moving brood throughout the nest (Cole 1994). New colonies may be relatively shallow and so may have less control over the extent to which the rearing temperature fluctuates with external temperatures. For example, 3-day-old lab *Pogonomyrmex rugosus* nests were on average 17 cm deep (Enzmann and Nonacs 2010) and mature *P. rugosus* colonies can be as much as 4 m deep (MacKay 1981).

The extent to which the first larvae experience food scarcity is also unclear. For colonies that are started claustrally, or without queen foraging, all the “food” for new larvae comes from the queen in the form of trophic eggs. These may or may not be scarce and may or may not be of high or low quality relative to the seed and dead insect forage that probably constitutes the diet of future broods. For queens that found colonies semi-claustrally, or with foraging, food availability for the brood depends on the queen’s foraging success as well as the quality and quantity of tropic eggs that she produces.

It is of course also likely that individual larvae vary genetically in ways that affect their final size. They may have differential ability to demand food based on their metabolic rates (Cassill and Tschinkel 1995). An effect of genotype or patriline on worker size was suggested by a study of *Acromyrmex echinatior* (Hughes et al. 2003). However, this study did not measure colony size, a potential confounding effect on the relationship between patriline and worker size distribution.

CONSEQUENCES

There are two levels of plasticity in the phenomenon of nanitics. First, there is developmental plasticity at the level of the individual worker. As discussed above, individual worker final size depends on the rearing environment of the larva, so one genotype can give rise to a variety of sizes of adult worker, or even a queen. Plasticity can also be seen at the level of the colony. Production of nanitics and then progressively larger workers with colony size represents a change in a colony-level phenotype, the colony's worker size distribution. In this section, I ask whether the individual-level and colony-level plasticity is likely to be adaptive at the new colony stage. What are the consequences of the production of small workers for colony performance?

There are many potential fitness consequences of the natural ontogenetic change in worker size. Workers of different sizes may differ physiologically and behaviorally. First, nanitic fire ants develop more quickly than do workers produced later by the same colony, and it may benefit new colonies to have

workers quickly (Porter 1988). Second, a new queen can presumably produce more nanitics than she can larger workers in her first clutch. Colony size is known to contribute to colony growth (Porter and Tschinkel 1986), so queens with larger first clutches should grow faster. Worker size may also affect colony growth directly via variable performance of workers of different sizes at brood care. Larger *S. invicta* produce brood at a lower rate than do smaller workers (Porter et al. 1985).

Worker size may also affect worker performance in colony defense. Sting length and total venom content increases with worker size in fire ants (Haight 2010). Also, a greater proportion of the colony's responding workers were majors when the attack was vertebrate-like vs. invertebrate-like, suggesting some worker size-related behavioral variation that could affect the extent to which the natural plasticity of the worker size distribution is adaptive (Haight 2010).

Worker size may also affect the ability of a colony to obtain food. In harvester ants, foraging costs colonies mainly in time (finding and retrieving). If a colony's foraging success is the mass of seed retrieved per unit time, worker size does not appear to affect colony foraging success directly; the size of seeds retrieved did not correlate with body size of *Veromessor pergandei* foragers (Rissing 1987). For desert ants, foraging can be dangerous because of the environment's combination of aridity and heat. Desiccation resistance itself may be affected by worker size; in many organisms, larger size reduces water loss (Lighton and Feener 1989; Chown and Gaston, 1999).

Colony success also probably also depends on energetic efficiency.

Production and maintenance costs may differ among workers of different sizes (Tschinkel 1988). Smaller fire ant workers are more expensive per gram in terms of production and maintenance combined (Calabi and Porter 1989). Perhaps because of these differences among workers in energetic requirements, a colony's ability to maintain its mass can depend on its worker size distribution. Ant colonies with more variably sized workers maintained their masses better in the face of food scarcity (Rising 1987, Billick 2007). In bees, worker size may relate inversely to starvation-resistance; in lab *Bombus impatiens* colonies starved for 10 days, dead workers were regularly removed, and thorax width of dead individuals decreased with the duration of starvation (Couvillon and Dornhaus 2010).

1.3 The study species

The genus *Pogonomyrmex* consists of sixteen species grouped into five species complexes (Strehl 2005). I studied *Pogonomyrmex barbatus* and *Pogonomyrmex occidentalis*, part of the *barbatus* and *occidentalis* species complexes, respectively. *P. barbatus* span the Southwest US (Arizona, New Mexico, Texas) and Mexico. *P. occidentalis* extend to higher latitudes and altitudes, in Colorado, Kansas, Nebraska, Utah, Wyoming, and Idaho (reviewed in Johnson 2000). These species share much of their ecology, differing on just a few points. Colonies of both species always have only one queen. Queens can live for decades (Keeler 1993). This means that though individual workers may die and be born, a colony can also live for more than a decade (Gordon 1991). Queens

of both species found colonies independently, without workers. The colonies then grow in number of workers until maturity, when they begin to produce winged reproductive males and females in addition to workers. The reproductives are released synchronously by the colonies in a population, each individual queen mating with multiple males (Wiernasz et al. 2004). In many populations formerly thought to be *P. barbatus*, not including the *P. barbatus* population that I studied, queen vs. worker caste is determined genetically (Helms Cahan et al. 2004). In some *P. occidentalis* populations, queens forage during founding (Wiernasz and Cole 2003), but *P. barbatus* queens are always claustral, feeding the first brood solely off of their own body reserves (McCook 1897).

Mature *P. occidentalis* and *P. barbatus* colonies contain approximately 20,000 and 10,000 workers, respectively (MacKay 1981, Keeler 1993). For the first year of life, colonies of both species are likely under 200 workers. Average-sized adult workers have an intrinsic lifespan of about a year. Workers of both species are continuously polymorphic, in contrast to their congener, *P. badius*, which has distinctly small (minor) and distinctly large (major) workers. That the mean and variance in worker size increases with colony size in *Pogonomyrmex* has only been established empirically for *P. badius* (Tschinkel et al.1998).

As the new colonies grow, the nest grows. The nest deepens with colony size (up to 3 meters for *P. barbatus*) and the vegetation immediately surrounding the nest is progressively removed. A mound tops *P. occidentalis* nests and grows with those colonies. Adult workers move brood along thermal gradients within this mound throughout the day (Cole 1994). Adult workers forage seeds, nectar

and recently-dead insects which they feed to the larvae (McCook 1897). Some nutrients are cycled out of the larvae as excretions which are ingested by the colony's adults (Wheeler 1994).

In both species, survival rates increase with colony size (Wiernasz and Cole 1995, Gordon and Kulig 1996). In *P. occidentalis*, colony size is known to correlate with colony growth rate and foraging success (Wiernasz and Cole 1995, Cole et al. 2010), and colony growth rate is correlated with mating frequency (Cole and Wiernasz 1999).

1.4 Research objectives

I aimed to discover both proximate and ultimate causes of the widespread phenomenon of nanitics, or production of extremely small workers by new social insect colonies that are founded independently. The experiment described in Chapter 2 revealed that the isolation of *Pogonomyrmex occidentalis* queens during colony founding limits both the size and number of their first worker offspring. This is a proximate answer to why new colonies make small workers. In Chapter 3, I describe an investigation that revealed a greater consequence of initial worker number than initial worker size to early colony growth, a proxy for fitness. This is an ultimate answer to why new colonies make small workers. Chapter 4 consists of a study of consequences of worker size for colony growth at a higher level of temporal resolution (production of a single batch of workers in a few weeks vs. the multiple batches over 8 weeks of the experiment in Chapter 2). That study was inconclusive but generally did not support the idea that

colonies pay high costs in brood care performance by producing nanitics. This study was meant to be a deeper look into the ultimate causes of a colony's worker size distribution. I explored the consequences of reproductive pattern (initial worker size and number) for colony success in another context (competition with other new colonies) in Chapter 5. I found no clear role for worker number but a possible role for queen size in biasing the outcome of a conflict. I conclude this dissertation with a summary and synthesis of my findings. Overall my results are consistent with the hypothesis that independently founded colonies benefit from making a first brood of very small workers. My results also suggest that a transition to production of larger workers with increased colony size is not only proximately somewhat inevitable, but also may benefit the colony during the rest of its potentially decades-long life.

2 Limits on reproduction during colony founding

Note: This chapter is a joint effort between myself and Diane Wiernasz. I did univariate analyses on the data, and then it was decided that a multivariate approach was more appropriate. Diane performed those multivariate analyses. She also contributed much of the introduction and added to the discussion.

2.1 Summary

1. Social insect colonies that are started by a single queen without adult workers make very small first workers. Colony founding conditions, such as food availability, temperature, and the number of adult caretakers, may contribute proximately to the size of these individuals.
2. I performed a full factorial experiment with two levels of each environmental variable (food, temperature, and care), testing for independent and synergistic effects on reproduction by newly-mated, field-collected *Pogonomyrmex occidentalis* (harvester ant) queens.
3. The first worker appeared sooner at higher temperatures and later when caretakers were supplemented. Food did not affect the time of first worker appearance. The queen's condition upon appearance of the first worker depended on food, temperature and the presence of adult workers during founding. Caretakers ameliorated an overall negative effect of low food on queen mass maintenance. It was mainly

in the absence of supplemental caretakers that queens in low temperatures lost more mass than did queens in high temperatures. Queens produced larger clutches at high temperature and supplemented care. Food on its own did not affect clutch size. The average size of worker in the first brood increased by 50% when care was supplemented. Food on its own did not affect average worker size. Queens produced larger workers at higher temperatures. There were trends towards interactions between food and care for worker size and clutch size.

4. The results suggest that new, independently founded social insect colonies may produce small workers proximately because the queen is alone in rearing the first clutch. The natural founding condition of solitude likely limits queens in their reproductive output and self-maintenance during colony founding. Food scarcity and low temperatures during colony founding may also limit queen maintenance and reproduction, respectively.

2.2 Introduction

Few phenotypes are more central to individual fitness than body size (Bonner 2006). Selection, operating through survival, mating success and fecundity variation, favors larger individual size across a wide range of organisms, including insects, vertebrates and plants (Kingsolver & Pfennig 2004). Directional selection favoring larger size may be constrained by opposing selection acting on

genetically correlated traits. Within species, large size is usually positively correlated with the length of the developmental period. If selection favors rapid development, balancing selection may lead to intermediate body size (Roff 2002).

Body size is a remarkably plastic trait in insects (Whitman & Ananthakrishan 2009). Many species display variation in body size across their geographic range (e.g. Mousseau 1997, Nylin & Gotthard 1998). In both herbivores and parasitoids, both body size and clutch size may differ among host species, reflecting effects on larval nutrition or the total amount of resources available for larval development (Awmack & Leather 2002). Studies of adaptive size variation in insects have focused on solitary species, where the developmental mechanisms responsible for body size variation that results from variation in the proximal environment are known.

In both natural and laboratory populations, insect reproductive patterns vary with aspects of the proximate environment. Many solitary holometabolous insects reach greater final sizes when reared in the lab at high food availability and lesser final sizes when reared in the lab at high temperatures (Nijhout 1981; De Moed et al. 1997; Davidowitz et al. 2004; Nijhout et al. 2006). These insects also usually lay larger clutches at higher temperatures and food availabilities (Dlussky & Kupianskaya, 1972; Eliopoulos & Stathas, 2005; Steiger et al. 2007; Berger et al. 2008).

The extent to which these mechanisms operate in social insects is currently unknown. Unlike the offspring of most solitary holometabolous species

which are deposited as eggs and feed themselves, the young of social insects are altricial and require extensive adult care. Workers who care for the eggs, larvae, and pupae may modulate both size and rate of development by altering the rate at which the young are fed (Cassill & Tschinkel 1999; Seeley 2009), the temperature at which they develop (Cole 1994; Seeley 2009), and potentially, via grooming, the rate at which they process food. Variation in the social environment represents another mechanism for producing variation in body size.

Although the process of colony founding is quite diverse, in a large number of social insect species, especially ants, new colonies are initiated by solitary foundresses (Holldobler & Wilson 1990). A mated queen digs a nest and raises the first brood of workers on her own. She may provision the immature brood entirely from her own reserves, including storage proteins and histolyzed flight muscle (Wheeler & Buck 1995; Wheeler & Martinez 1995, Hahn et al. 2004), or she may forage for additional food outside the nest. These first workers ("minims" or "nanitics") are extremely small relative to the typical worker in an older colony. The increase in worker body size as the colony matures may reflect larger numbers of caregivers, larger amounts of food, and a more homeostatic thermal environment (Cole 1932; Brian, 1957; Lavigne, 1969; Tschinkel, 1988; Cole 1994; Enzmann & Nonacs 2010). If workers of larger size are evolutionarily favored because they increase colony performance, the small size of early workers may reflect constraints in the rearing environment of newly founded colonies.

To address this gap in our understanding, of the proximate causes of nanitics, I examined the role of variation in the external environment (temperature, food) and in the social environment (presence of workers) in shaping the development rate and size of workers in newly founded colonies of the harvester ant, *Pogonomyrmex occidentalis* (Cresson). The natural covariation among environmental factors and reproduction in social insect colonies makes it hard to identify a causal relationship between a specific factor and an aspect of reproduction using natural populations. By manipulating these parameters in colonies reared in the laboratory, I assessed both the direct effects and interactions of food, temperature and care on multiple aspects of colony reproduction: time until appearance of the first adult worker, queen condition at appearance of the first worker, offspring number, and offspring size.

I made predictions based on solitary insect developmental biology (Table 2.1). I expected that both increased food availability and higher temperatures would decrease the time to the first adult worker and increase queen condition and offspring number. Additionally, I expected higher food levels to lead to larger offspring. Given the pattern of increasing worker size through colony ontogeny, I expected caretaker supplementation to decrease time to the first worker and increase queen condition, offspring size, and offspring number.

Table 2.1 Predicted effects of environmental factors on new colony traits

Factor	Time to first worker	Queen condition	Offspring size	Offspring number
food	↓	↑	↑	↑
temperature	↓	↑	↓	↑
care	↓	↑	↑	↑

Upward arrows indicate a predicted positive relationship between the level of the environmental factor and the colony trait in question. Downward arrows indicate a predicted negative relationship.

2.3 Materials and Methods

STUDY ORGANISMS

191 *P. occidentalis* queens were collected near the site of a long-term field study in western Colorado (Wiernasz & Cole 1995). Queens in this population found colonies solitarily and forage before the first workers appear (Billick et al. 2001). Queens were collected on three days after a mating flight that occurred on 13 July 2010, packaged individually with damp cotton into plastic vials, and refrigerated at 5^{o}C until they were shipped overnight to the laboratory at the University of Houston.

STUDY DESIGN

Upon arriving in Houston, queens were placed individually into 17 x 12 x 6 cm clear plastic boxes containing a 20 x 150 mm test tube partially-filled with water and plugged with a cotton ball. Colonies were maintained in incubators unless they were being fed or measured. Queens were arbitrarily assigned to one of eight combinations of food (high/low), temperature (high/low), and supplemental

workers (added/not added) (Table 2.2). Colonies received three types of food: honey water, seeds, and cricket pieces. Each colony received a wad of honey water soaked Kimpak, which was changed weekly. Low and high food colonies differed in the frequency and quantity of seeds and cricket pieces that they received. Low food colonies were fed approximately 50 mg of seeds (sunflower seeds and cracked wheat) every third week for the first six weeks and then once more 11 weeks later. These colonies received approximately 30 mg of cricket in the form of a cricket leg every week, beginning in the third week of the experiment. High food colonies initially received approximately 180 mg of seeds every 7-14 days (Table 2.3), but by the tenth week of the experiment, seeds were almost covering the floor of the boxes containing these colonies. High food colonies were then fed seeds two weeks later and then not again until the final week of the experiment. High food colonies received approximately 140 mg of cricket weekly. The seeds in both high and low food treatments never completely disappeared between feedings, but the cricket leg in the low food treatment was scraped clean by the ants each week.

	Treatment								
	low food low temp independent	low food low temp caretakers	high food low temp independent	high food low temp caretakers	low food high temp independent	low food high temp caretakers	high food high temp independent	high food high temp caretakers	Total
Assigned	24	21	24	22	25	23	25	22	186
Dead pre-emergence	7	4	4	0	5	0	2	1	23
No offspring	0	0	0	1	0	0	0	0	1
Produced queen	0	0	0	0	0	1	0	0	1
	17	17	20	21	20	22	23	21	161
Offspring omitted	5	0	0	0	0	2	0	0	7
	12	17	20	21	20	20	23	21	154
No queen initial mass	1	0	0	0	0	0	0	0	1
	16	17	20	21	20	22	23	21	160

Table 2.2. Of the 191 queens collected, 186 survived the 24 hours between arrival in Houston and treatment assignment. 23 more queens died before producing an adult. One queen did not have any offspring in the entire 5 months of the experiment. Another queen produced a female reproductive (gyne) as part of its first clutch. This colony's reproduction was omitted from analyses because (1) gyne production as part of the first clutch is probably rare in nature and so this colony is considered an anomaly and (2) gynes are massive and so would constitute a major fraction of a queen's reproduction, skewing offspring number and total offspring mass. This left 161 queens included in the analyses of time to the first worker and offspring number. I mistakenly didn't weigh all the offspring of the first clutch of 7 colonies. This left 154 colonies included in analyses of total mass of the first clutch and offspring size. I mistakenly did not take the initial mass of one of the 161 queens that produced only worker offspring during the experiment, so only 160 queens were included in the analyses of proportion of queen mass lost.

Table 2.3: Food administration log

	Treatment					
	high food			low food		
date	cricket	seeds	honey water	cricket	seeds	honey water
7/16	0.33	small pinch mixture	1	0	0	1
7/23	0.33	0	1	0	0	1
7/30	0.5	small pinch mixture	1	1 leg	2 cracked wheat particles, 1/2 sunflower seed	1
8/6	0.5	small pinch mixture	1	1 leg	0	1
8/13	0.5	0	1	1 leg	0	1
8/20	0.5	small pinch mixture	1	1 leg	2 cracked wheat particles, 1/2 sunflower seed	1
8/28	0.5	0	1	1 leg	0	1
9/3	0.5	small pinch mixture	1	1 leg	0	1
9/11	0.5	small pinch mixture	1	1 leg	0	1
9/17	0.5	0	1	1 leg	0	1
9/24	0.5	0	1	1 leg	0	1
10/1	0.5	small pinch mixture	1	1 leg	0	1
10/8	0.5	0	1	1 leg	0	1
10/15	0.5	0	1	1 leg	0	1
10/22	0.5	0	1	1 leg	0	1
10/29	0.5	0	1	1 leg	0	1
11/5	0.5	small pinch mixture	1	1 leg	2 cracked wheat particles, 1/2 sunflower seed	1

Half of the colonies were reared at 26°C (low temperature), and half were reared at 30°C (high temperature). In half the colonies, queens raised the initial brood alone, mimicking the normal founding conditions in this species. In the remaining colonies, queens were given supplemental workers in the form of pupae within a day of placing the queens in their lab nests. I removed worker pupae from older colonies present in the lab, scored their developmental stage based on pigmentation, and distributed them as uniformly as possible across colonies. The number of pupae that successfully eclosed ranged from 2 to 8.

I measured six aspects of colony reproduction: time to the first worker, queen condition at the appearance of the first worker, size (wet mass) of the first worker, average size (wet mass) of workers in the first clutch, number of workers in the first clutch, and total wet mass of the first clutch. Colonies were examined daily for the presence of new workers. Colonies with new workers were refrigerated at 8°C to immobilize the workers, who were then removed and individually weighed on an analytical balance (AT20 Mettler Toledo, Ohio, USA) to the nearest 0.001 mg, and frozen.

The colony's first clutch was defined at the eclosion of the first new worker (exclusive of any supplemented workers). At this time, the pupae in the colony were counted and this value was added to the number of adult workers present; this was considered the number of offspring in the first clutch (Kudô 2003).

At the start of the experiment, queens were refrigerated at 8°C for approximately 30 min and then weighed. They were weighed again when the colony's first worker eclosed. These measures were used to calculate the

queen's mass loss (queen initial wet mass - queen wet mass at first offspring) for each colony. Queen mass loss was regressed on queen initial mass and the residuals were used in the multivariate analysis described below.

STATISTICAL ANALYSES

All analyses were performed in Systat 11 (Wilkinson 2004). Some colonies were mistakenly left out of the incubators (in the refrigerator or on the benchtop) overnight after a new worker appeared. Most results were unaffected by exclusion of these colonies. Where exclusion of these colonies from analyses changed the overall results, results are reported both with and without those colonies. Thirty-eight out of the 191 collected queens constituted other special cases (such as early queen death) and were omitted from some or all of the analyses (Table 2.3). I first estimated the correlations among the dependent variables. Two of these (the mass of the first brood, the size of the first worker to eclose) were highly positively correlated with two of the other dependent variables (size of the first brood, average size of workers in the first clutch). I restricted the analysis to the four remaining variables. Time to eclosion of the first worker, average worker size, and clutch size were log-transformed to produce normal distributions. To test for an effect of queen size, I regressed three variables (time to the eclosion of the first worker, size of the first brood, average size of workers in the first brood) on queen initial wet mass (queen wet mass within a day of mating). None of the regressions was significant, so queen initial mass was not included in further analyses.

Although I removed highly interdependent variables from the study, the remaining measures were expected to be interdependent to some (unknown) degree. I used multivariate analysis of variance (MANOVA) to test for the effects of my treatment manipulations. Temperature, food and supplemental workers were main effects; the first two were categorical, but I used the number of surviving supplemental workers as the caretaker effect (range from 0 = no addition, 2-8 additional). I first tested for an interaction between all main effects, but found no significant difference for any of the variables. I then tested the three two-way interactions, and found that there was virtually no difference for any of the variables for the interaction between temperature and food. My final model included all three main effects and two of the two-way interactions (caretakers x food, caretakers x temperature).

2.4 Results

Overall, the MANOVA was highly significant (Wilk's lamda = 0.0673, $F_{4,146} = 445$, $P << 0.001$). All factors tested, including interactions, were significant overall. The full results of this analysis are presented in Table 2.5. Below I highlight the most important outcomes.

Table 2.4: Multivariate results

Factor	Wilk's lambda	DF	*F*	P
temperature	0.249	4/146	110.1	< 0.001
food	0.831	4/146	7.4	< 0.001
caretakers	0.366	4/146	63.3	< 0.001
caretakers*temperature	0.909	4/146	3.7	0.007
caretakers*food	0.923	4/146	3.1	0.019

TIME TO ECLOSION OF THE FIRST WORKER

Temperature significantly affected the time to the first worker ($F_{1,149}$ = 369.64, P <<0.001). Queens maintained at 30°C produced a first worker approximately 20 days earlier than did queens maintained at 26°C. Although of much smaller magnitude, time to the first worker was also affected by care ($F_{1,149}$ = 4.51, P = 0.035). Queens with supplemental workers produced a first worker approximately a day later than did queens which reared brood alone. Food did not affect time to the first worker ($F_{1,149}$ = 1.41, P = 0.236) (Figure 2.1).

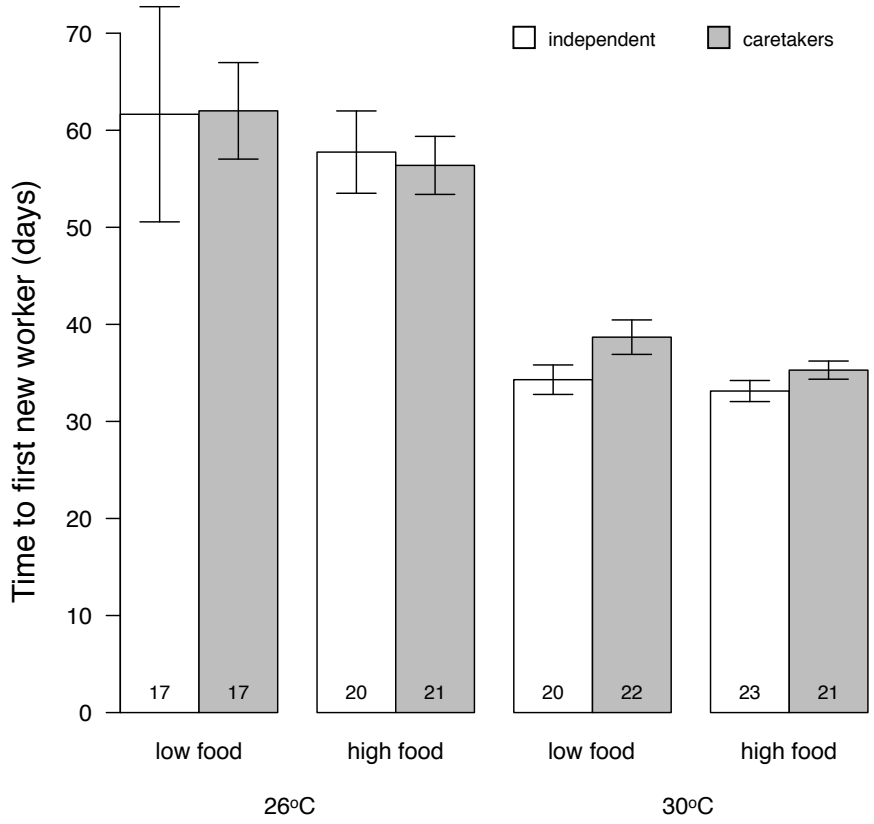

Figure 2.1: Time to first worker. Bars and whiskers are means and 95% confidence intervals, respectively, of the time to the first worker. Each bar represents a treatment combination (e.g. bar 1 is for independent queens whose colonies were reared at low food and low temperature). The value within a bar is the number of replicate colonies included in the analysis.

CLUTCH SIZE

Colonies produced between one and sixteen workers totaling to 1.8 to 73.0 mg of offspring in their first clutches. The number of workers in the first clutch was significantly affected by the presence of supplemental workers ($F_{1,149} = 60.58$, $P < <0.001$) and marginally by temperature ($F_{1,149} = 3.87$, $P = 0.051$), but not by food ($F_{1,153} = 1.16$, $P = 0.283$) (Figure 2.2). Worker-supplemented queens made twice as many offspring as solitary queens. There was a trend towards an interaction between food and care ($F_{1,149} = 3.40$, $P = 0.067$). Food on its own did not affect clutch size, but caretaker-supplemented colonies produced larger clutches with high food than with low food.

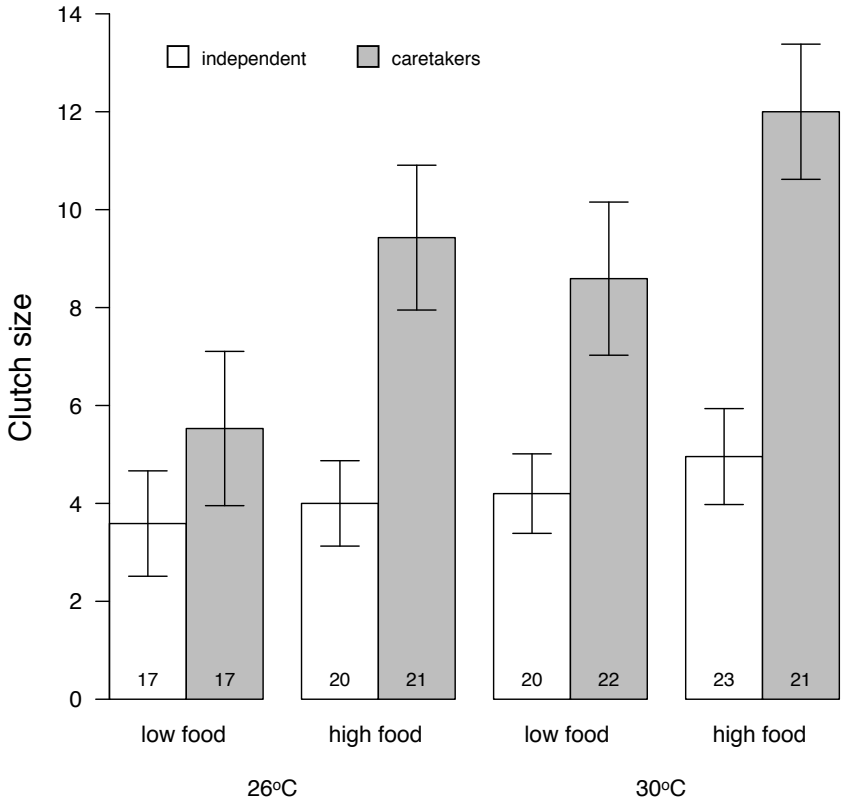

Figure 2.2: Clutch size. Bars and whiskers are means and 95% confidence intervals, respectively, of the number of offspring in the first clutch.

AVERAGE WORKER SIZE

Average worker size was most strongly affected by care ($F_{1,149}$ = 193.32, P << 0.001) (Figure 2.3). Queens with additional workers produced offspring that were 50% larger than those of independent queens. Food on its own did not affect average worker size significantly ($F_{1,149}$ = 1.97, P < 0.163). There was a trend towards an interaction between food and care. Care-supplemented colonies reared with high food produced smaller workers than did colonies reared with low food. Average worker size did respond to temperature ($F_{1,149}$ = 10.01, P < 0.002). Queens produced workers that were 11% larger at high vs. low temperature.

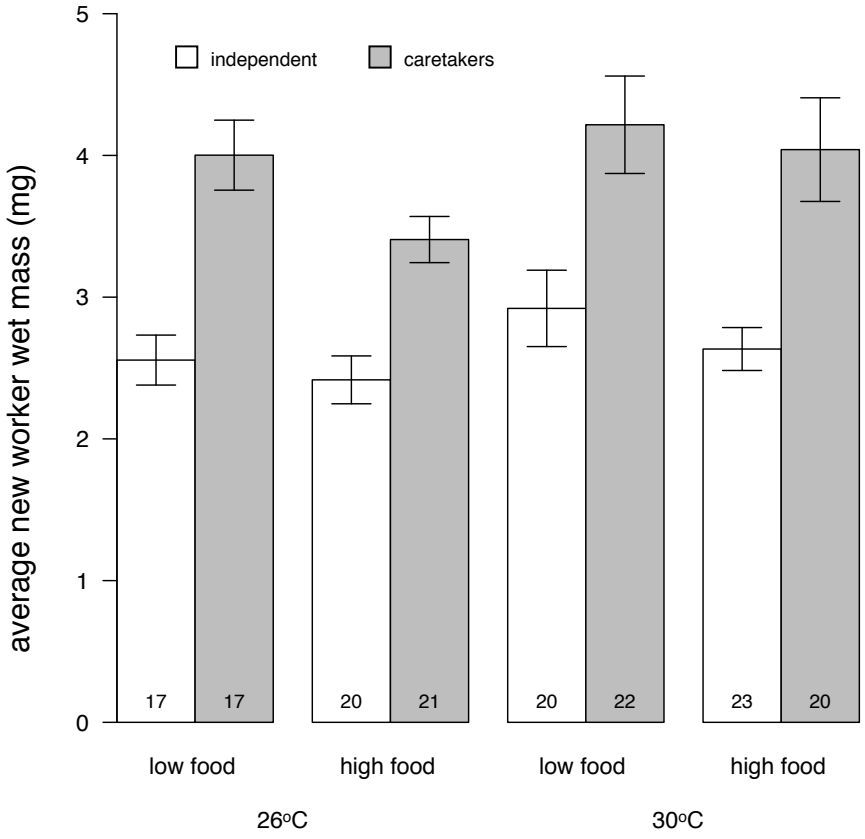

Figure 2.3: Average size of new worker in the first clutch. One colony with missing data was excluded from the analysis and the graph.

QUEEN CONDITION

Food ($F_{1,149}$ = 22.55, P << 0.001), temperature ($F_{1,149}$ = 13.78, P = 0.0003), and the number of caretakers ($F_{1,149}$ = 13.25, P = 0.0004) affected the queen's condition when the first worker eclosed (Figure 2.4). Queen mass loss was 50% lower under high food compared to low food. Queens reared at high temperature lost only one-third of the mass lost by queens reared at low temperatures. Queens with supplemental workers lost half the mass lost by independent queens. There was an interaction effect of temperature and worker supplementation ($F_{1,149}$ = 6.95, P = 0.009). It was mainly in the absence of supplemental caretakers that queens in low temperatures lost more mass than did queens in high temperatures. There was also an interaction effect of food and worker supplementation ($F_{1,149}$ = 5.56, P = 0.020). Absence of supplemental caretakers exacerbated the negative effects of low food on queen mass maintenance. Queens generally lost more mass at low food than at high food, but when caretakers were present, the difference in queen mass loss between low and high food environments was not as great.

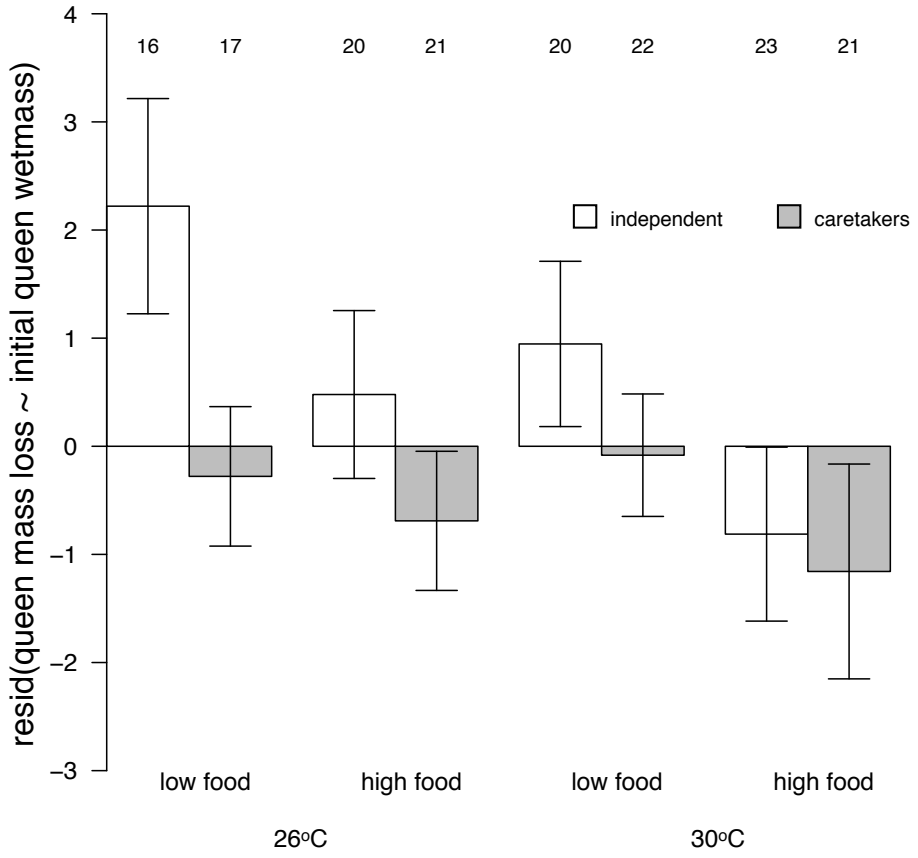

Figure 2.4: Residuals of a regression of queen mass loss on queen initial mass. One queen with missing data was excluded from the analysis and graph.

2.5 Discussion

The results reveal both similarities and differences among new social insect colonies, mature social insect colonies, and non-social insects in responses to the environmental parameters of food availability and temperature. Most of the main effects of the environmental variables on reproduction were in the direction expected based on non-social insect biology (Table 2.1). First, temperature usually increases non-social insect larvae development rate, and in this experiment, workers from new ant colonies emerged sooner in colonies at 30^{o}C

than at 26^{o}C. Offspring of another ant species, *Linepithemia lumile*, also develops more quickly at higher temperatures (Abril et al. 2010).

Second, under favorable conditions, parents generally use less of their own resources for reproduction. Queens retained a greater proportion of their initial mass upon eclosion of the first worker under higher food, higher temperature, and supplemental care. These conditions can be considered "favorable", reducing the costs of reproduction to the queen. Queens of semi-claustral harvester ant species (*Pogonomyrmex californicus* and *P. desertorum*) that were provided with food retained more mass during colony founding than queens that were unfed (Johnson 2004). Queens of some species, including *P. californicus*, sometimes cooperate and found colonies jointly. When *P. californicus, Atta texana* (texas leafcutter ant) and *Solenopsis invicta* (fire ant) queens co-found colonies with more queens, they lose less mass (Mintzer 1987; Tschinkel 1993; Bernasconi & Strassmann 1999; Johnson 2004). These results suggest that under natural conditions, the extent to which *P. occidentalis* queens must invest their own resources into brood production will depend on their success as foragers, and their size. Foraging success by the queen may also limit the size of the first clutch. In this experiment, where all food treatments may have represented relatively high levels of food, I did not observe a significant relationship between queen size and clutch size.

Third, for organisms that care for their young, more care usually translates into greater offspring numbers, greater offspring sizes, and greater total mass of the first clutch. The present results fit this expectation— colonies supplemented

with caretakers produced larger and more offspring than did those with a single caretaker. Bigger colonies make bigger workers in *Pogonomyrmex badius* (Florida harvester ant), *S. invicta* (fire ant), and *Myrmic rubra* (European red ant)) colonies, (Brian 1973; Wood & Tschinkel 1981; Porter & Tschinkel 1985).

Surprisingly, increased food availability did not increase reproduction. A positive relationship between food availability and clutch size exists for other insect species: *Venturia canescens* (parasitoids), new *Polistes chinensis* (paper wasp) colonies, new *P. californicus* colonies, and new *P. desertorum* ant colonies (Howard & Jeanne 2004; Johnson 2004; Eliopoulos & Stathas 2005), and was expected in this experiment. One reason I may not have found an effect of food is that the low food treatment may have represented a relatively large amount of food for this species. Compared to claustral congeners (e.g., *P. barbatus*), queens of *P. occidentalis* are relatively small, and actively forage throughout the initial stage of colony founding. The daily food intake of foundress queens may be significantly lower than what was available to low food colonies in this experiment.

One environmental effect on reproduction that could not be predicted from solitary insect biology was the effect of caretaker supplementation. Since workers generally facilitate the production of new workers, worker supplementation was expected to accelerate larval development. My results do not match this prediction perhaps because larvae reared by workers received more food per capita, or were fed more often, than those reared by solitary queens. In holometabolous insects, developmental time is set in part by the critical size (size

at which larvae pupate) which is in turn set by food availability early in larval development (Davidowitz et al. 2003). Larvae receiving less food or less frequent feeding may set a lower critical size and so had to grow less to reach adulthood. There is also a potential ultimate explanation for the increased time to the first worker for caretaker-supplemented colonies. The priority of fast larval development might diminish as a colony grows. Accordingly, new colonies with more workers may start to siphon more resources into production of larger offspring growth, rather than ones that develop rapidly. This could lead to the observed production of larger workers by caretaker-supplemented colonies and the increase in time to the first worker. The results of the present study contrast with those of a study of the effects of colony size on time to the first worker in a swarm-founding wasp, *Polybia occidentalis*. Howard and Jean (2004) found that field colonies that naturally started with greater worker populations produce a new worker more quickly than those started with fewer workers. The field colonies that naturally started with more workers may have been those with higher quality queens, however, so a causal relationship between worker number and time to first worker cannot be drawn from that study. Larger queenless fragments of mature lab colonies of *M. rubra* rear workers more quickly (Brian 1953), but the extent to which this relationship between adult worker number and new worker development rate is applicable to new colonies is unknown.

Interactions between the worker treatment and the abiotic variables were expected because the physiology and behavior of workers may change in response to food availability and temperature, and these interactions were

observed. First, clutch size was higher with additional workers and high food. This could be because workers are more efficient at converting food into offspring.

A full explanation of the low levels of reproduction in natural new colonies will require an investigation of the fitness effects of a particular reproductive pattern, specifically particular worker sizes, on new and mature colonies. Given the extremely low survival rates of new *P. occidentalis* field colonies (Wiernasz & Cole 2003), it would be interesting to know the extent to which worker size and clutch size affect colony survival. New fire ant colonies with more and smaller workers grow more quickly (Porter & Tschinkel 1986), despite small workers having lower per capita task efficiency (Brian 1953). Colony growth rate is known to affect the survival probability of young colonies in the field (Cole & Wiernasz 1999), so selection may indeed favor worker number over worker size in new colonies. Offspring size and number may also impact colony fitness in other ways. For example, worker size affects foraging efficiency in some wasps (Jandt et al. 2010).

These results highlight the importance of the social environment in determining the pattern of reproduction of new social insect colonies. In this experiment, worker supplementation increased offspring size tremendously, suggesting that larvae in new natural colonies receive poor care, such as infrequent provisioning. This hypothesis could be tested through direct observation of rates of larval provisioning in colonies of different sizes and ages. Natural patterns of social insect colony growth are to some degree products of

the larval rearing environment. Food availability and temperature affect colony establishment, and colony composition (number of workers) can magnify these effects.

Table 2.5: Effects of experimental treatments on colony response variables

Factor	DF	*F*	P
Time to first worker			
temperature	1/149	369.6	<0.001
food	1/149	1.4	0.236
caretakers	1/149	4.5	0.035
caretakers*temperature	1/149	4.2	0.430
caretakers*food	1/149	1.2	0.284
Clutch size			
temperature	1/149	3.9	0.051
food	1/149	1.2	0.283
caretakers	1/149	60.6	<0.001
caretakers*temperature	1/149	0.35	0.553
caretakers*food	1/149	3.4	0.067
Average new worker size			
temperature	1/149	10.0	0.002
food	1/149	2.0	0.163
caretakers	1/149	193.3	<0.001
caretakers*temperature	1/149	0.1	0.741
caretakers*food	1/149	3.2	0.076
Queen mass loss			
temperature	1/149	13.8	0.003
food	1/149	22.6	<0.001
caretakers	1/149	13.3	<0.001
caretakers*temperature	1/149	7.0	0.009
caretakers*food	1/149	5.6	0.020

Acknowledgements
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3 First clutch size matters for social insect colony growth

3.1 Abstract

Clutch size and offspring size affect fitness in many organisms. In social insects, there may be a trade off between clutch size and offspring size at two levels of reproduction. One kind of reproduction is production of the first brood of workers by new queens. There may be a trade-off between the size and number of workers that new queens can produce in the first brood. I measured the consequences for the first 8 weeks of colony growth of first clutch size and of the size of workers in the first clutch of newly-mated *Pogonomyrmex barbatus* queens. I found that colonies of queens with greater first clutches grew faster and that worker size did not affect colony growth. These results are consistent with the idea that in new colonies, the size vs. number trade off in terms of worker production is resolved on the side of worker number for an advantage in colony growth. In social insects, selection is thought to act at the colony level, so these results suggest that selection may favor colonies that allocate their resources during alate production to produce colonies (offspring) of greater first clutches (initial size). This advantage of initial offspring size for colony growth is similar to previously observed advantages of seedling size to early plant growth.

3.2 Introduction

Faced with limits in the number and size of offspring they can produce in a single bout, organisms may allocate their resources among offspring number and size according to the relationships among those traits and parental fitness. Mothers with a set total amount of reproductive resources must divide available energy and nutrients among offspring size and number, but selection may favor both offspring size and number. Selection may favor the production of larger offspring if those offspring grow faster (e.g. seed size and seedling growth rate in plants reviewed in Westoby et al. 1992; egg size and feeding rate and larval development rate in flies: Azevedo et al. 1997), survive better (e.g. in lizards: Uller & Olsson 2010), or produce more and higher quality offspring themselves (e.g. in some insects: reviewed in Honek 1993; anoles: Cox & Calsbeek 2010). Selection may favor larger clutches of offspring because simply they *are* more offspring that may survive and themselves reproduce. Assuming fixed total maternal costs of reproduction, many organisms may face a trade-off between offspring size and clutch size.

Many social insect queens face a similar offspring size and number trade off at the start of their colony lives, when they produce their first clutch of workers. When a colony reproduces, it makes winged reproductive males and females, or alates, the females of which mate, disperse, and start a new colony by producing a first brood of workers. In many species, new queens, or foundresses, start colonies claustrally, without foraging and instead relying just on their body reserves for production of the first clutch of workers. Especially for queens that

found colonies claustrally, initial worker number and the size of workers in the first clutch may limit each other (i.e. selection on one may affect the other because they draw from common resources). Data from experiments with *Pogonomyrmex occidentalis* support this idea (Appleby & Wiernasz, unpublished). Colonies in many social insect species are started independently, by queens without adult workers (vs. dependent swarming/budding) and these colonies universally make a first clutch of extremely small workers, nanitics (summarized in Wood & Tschinkel 1981).

The consequences of investment in first clutch size vs. initial worker size for queen fitness have been studied in fire ants. In new fire ant colonies, workers brood-raid—they enter neighboring nests, pick up larvae and/or pupae and carry them back to their own nest. Sometimes, simultaneously, workers from the raided nest raid the other colony, so the brood are carried back and forth between the colonies (Tschinkel 1992). In fire ants and other species that brood raid, then, it seems that there could be an additional advantage to rearing first broods that are large in number, rather than individual worker size—colonies with more workers may be more likely to “win” or end up with more brood at the end of these brood raiding sessions. It is more energetically efficient, though, for colonies to grow via production of larger workers, because larger workers have lower metabolic rates and live longer (Calabi & Porter 1989). Larger worker size may also benefit colonies because it confers greater desiccation resistance to individual workers (Lighton et al. 1994, Hood & Tschinkel 1990).

Social insect colony fitness often depends on early colony growth rate and

early colony size. Juvenile mortality is high for colonies that are started independently, or by queens without adult workers. For example, only 5% of incipient *Solenopsis invicta* colonies survive to the juvenile colony stage (or through the first months of colony life) (Tschinkel 1992). In *Pogonomyrmex occidentalis*, less than 1% of marked foundresses survived to the juvenile colony stage (Wiernasz et al. 2003) This high juvenile mortality may be escaped by quick growth out of the juvenile stage; in field *S. invicta* colonies, for example, colony growth rate affects survival (Markin et al. 1973), and larger new fire ant colonies generally survive better (Markin et al. 1972).

A likely mechanism for first clutch size and initial worker size to affect colony growth is sibling care, a defining aspect of social insect colonies. In colonies of eusocial organisms, not one individual, but many, care for the offspring of one or a few fertile individuals. Having more caretakers may affect brood care by leading to more brood care acts (feeding, grooming) per larva and higher quality brood care acts per larva. Better brood care could in turn lead to better survival rates of early stage offspring that would otherwise die off or more rapid development of offspring. Just as initial clutch size could affect the quality and quantity of care received by the next brood, so could the size of the first workers. Larger workers may be able to process food more effectively (chemically and physically) and so might promote brood survival and accelerate brood development. Worker size and number both could also translate into better care for the queen and therefore a higher egg-laying rate. In fire ants, where there are major and minor subcastes of workers, the relative brood care

proficiency of large minor workers vs. nanitics was assessed. Larger minor workers were better brood care takers than nanitics, but larger colonies in terms of worker number produced more brood (Porter & Tschinkel 1986). By producing smaller workers in the first clutch, queens may be able to produce larger first clutches, in terms of worker number.

Data from field studies of harvester ants are consistent with the hypothesis of the adaptive value of nanitics, but the consequences of the size and number of workers in the first clutch have not been measured. Like fire ants, harvester ants also produce nanitics, but harvester ants have a single continuously-polymorphic worker caste. Harvester ants of the genus *Pogonomyrmex* have been the subjects of multiple long-term field studies at two sites, so much is known about their natural history. In a New Mexico population of *Pogonomyrmex barbatus*, nanitics are active above ground for approximate two months starting one month after the mating flight (Wagner & Gordon 1999) and young (1-2 year old) colonies compete with each other for foraging territories (Gordon & Kulig 1996). In a Colorado population of *P. occidentalis,* rates of survival of the incipient colony stage are low (Wiernasz & Cole 2003) and larger colonies survive better (Wiernasz & Cole 1995). Together, these results are consistent with the idea that nanitics are an adaptation for high initial colony growth rate (via a greater initial colony size than would be possible with larger first workers). Field study of nanitics is difficult because it is rare for queens to survive even just to the eclosion of their first broods and new colonies are relatively inconspicuous.

I set out to explicitly measure the consequences for colony growth of first clutch size and initial worker size. I collected newly-mated *P. barbatus* queens, manipulated their first clutch sizes and initial worker sizes, and censused the colonies for 8 weeks (past maturation of the first clutch). I found that first clutch size mattered for colony growth—colonies with larger first clutches grew faster.

3.3 Materials and methods

FIELD COLLECTION OF QUEENS AND EARLY REARING

I collected 186 *Pogonomyrmex barbatus* queens during and after a mating flight in Bear Creek Golf World in Katy, TX on June 17, 2012. I brought the queens to the lab, rehydrated them by giving them access to a water-soaked cotton ball for a few hours, and then weighed them on an analytical balance (AT20 Mettler Toledo, Ohio, USA) to the nearest 0.01 mg.

In nature, *P. barbatus* queens found their colonies claustrally; they produce the first batch of workers without foraging. So, after weighing each queen, I placed her in her own test tube filled partially with water and plugged with a cotton ball. I stored these tubes in a 30^{o}C incubator in constant darkness until the first workers eclosed. Starting two weeks after the mating flight, I checked the tubes weekly for mature (yellow) pupae.

When adult workers began to appear, on July 18, 2012, I counted each colony's pupae and larvae. In preparation for construction of the experimental first clutches, I removed the pupae from most of the colonies. In one set of control colonies (Control B), the pupae were removed from and later returned to

their original colonies. In another set of control colonies, the pupae were left untouched in the nest (Control A). There were five Control A colonies and ten Control B colonies.

MANIPULATION OF THE FIRST CLUTCH

I collected pupae from one-year-old stock colonies and pooled these with pupae collected from the new queens. Together, these pupae constituted the source for the new first clutches of the experimental colonies. These pupae were also used for a third control group (Control C). The 10 colonies in Control C had their own pupae removed and replaced, one-for-one, with foreign pupae.

I created four initial worker size treatments that varied in the mean size of worker in the first clutch. The four worker size treatments were small nanitics (pupae produced by newly mated queens), large nanitics, small non-nanitics (pupae produced by the one-year-old stock colonies), and large non-nanitics (Figure 3.1). To create these size treatments, I first sorted the source pupae according to size using sieves and by picking through them manually with forceps. I then quantified pupal headwidth by imaging the pupae facedown on an Epson Perfection V500 Photo scanner. I measured each pupa's headwidth, or distance from the outside edge of one eye to the outside edge of the other, to the nearest 0.01 mm with ImageJ.

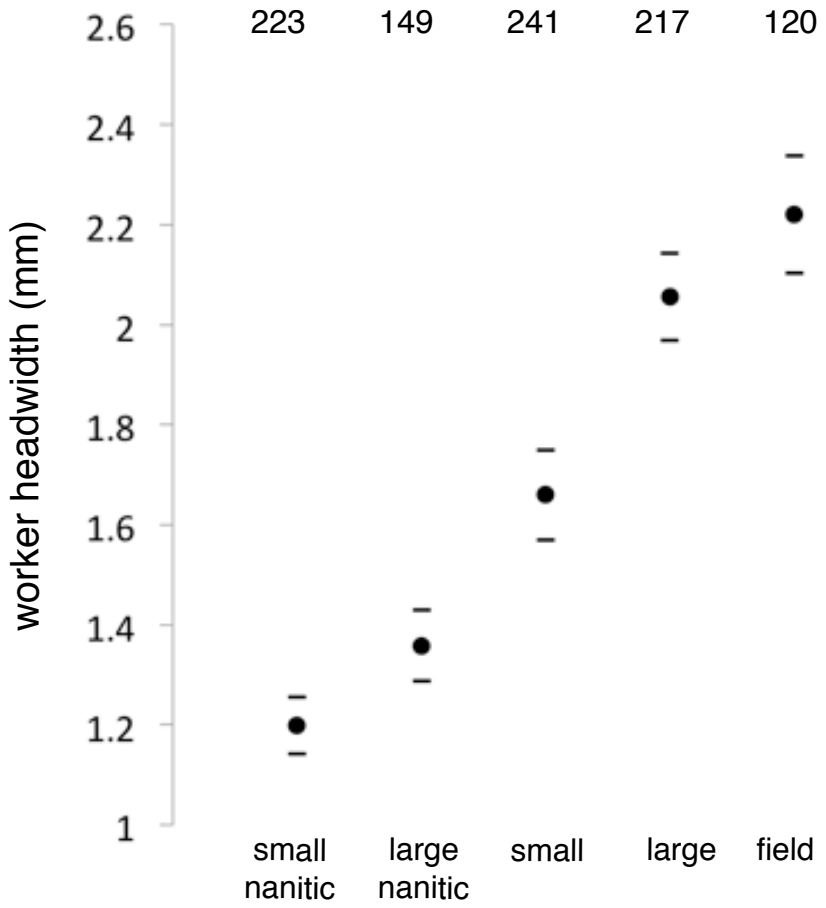

Figure 3.1: Worker size variation. Headwidths (mm) of workers placed in the experimental colonies (from left to right: small nanitic, large nanitic, small non-nanitic, and large non-nanitic). The right-most point and dashes are for workers collected from established field colonies. The point and dashes represent the mean and standard deviation, respectively. Workers collected from field colonies were measured by freezing them as adults, cutting off their heads, and sliding their heads into a wedge micrometer (Porter 1983). The values along the top are the numbers of individual workers measured of each type.

Pupae mature over the course of eight to sixteen days (Appleby, unpublished), so the collected pupae were of a range of ages. As pupae mature, they progress from being completely colorless to having pigmented eyes, and then to being yellow with pigmented eyes. I preferentially chose pupae that had reached or passed the colorless-body-with-dark-eyes stage. A few of the large

non-nanitic pupae included were still in the completely colorless stage. The dark-eyes requirement was also relaxed for the nanitic treatments because of dearth of supply.

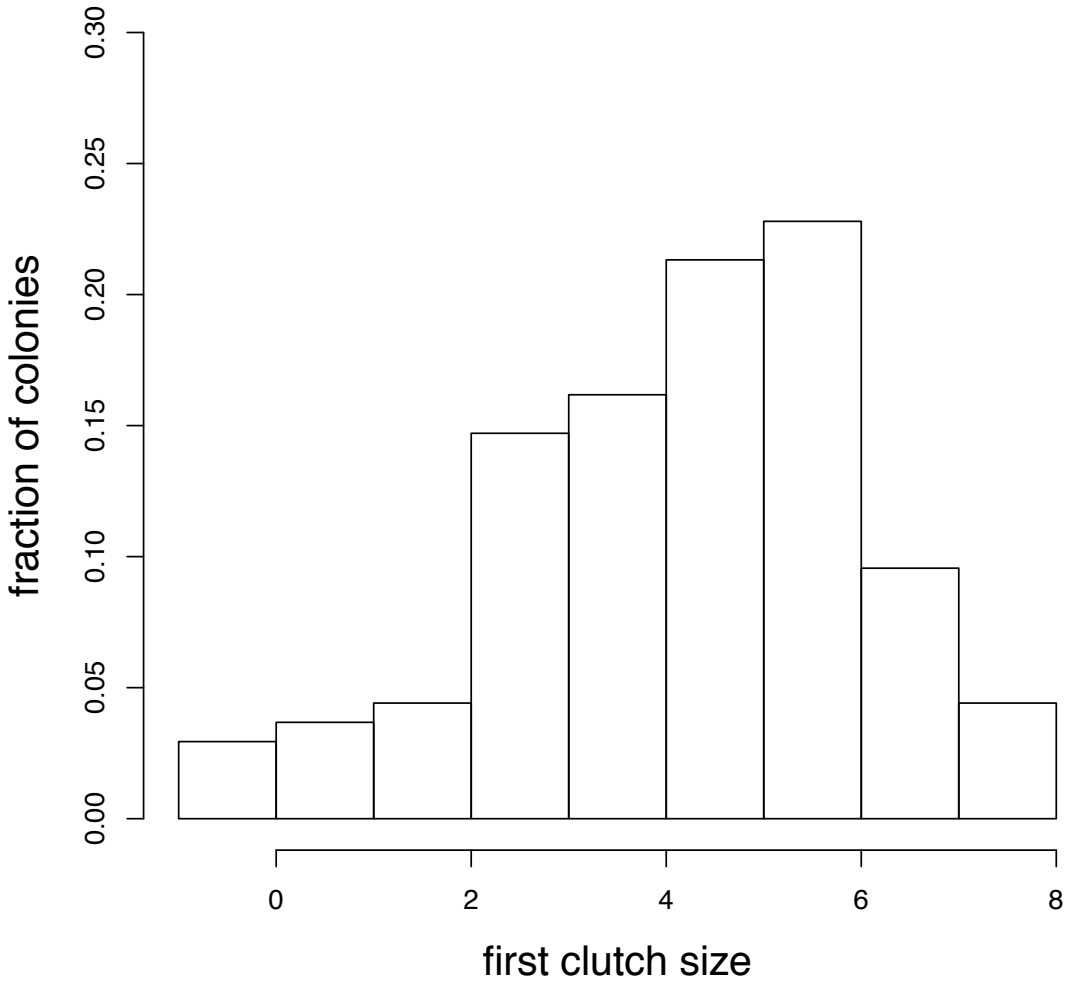

Figure 3.2: Natural first clutch size distribution. 136 newly-mated *Pogonomyrmex barbatus* queens were collected in the field and reared claustrally until just prior to worker eclosion. The number of pupae present in each colony at that time was assessed and is displayed here.

The natural variation in first clutch sizes in this population is quite limited (0-8, Figure 3.2), so I extended the phenotype for the first clutch size treatments, gathering the pupae into groups of one, five, or ten. Each group consisted of only

small or large nanitics, or small or large non-nanitics. Starting sample sizes were relatively invariant across experimental group (Table 3.1).

Table 3.1: Planned clutch size treatments

worker size	clutch size	replicate colonies
small nanitic	1	15
small nanitic	5	14
small nanitic	10	13
large nanitic	1	15
large nanitic	5	10
large nanitic	10	8
small	1	15
small	5	15
small	10	15
large	1	15
large	5	16
large	10	12

I placed each queen in a clear plastic ant box (17 x 12 x 6 cm) that had its sides coated with two layers of Insect-a-Slip Insect Barrier (BioQuip) to prevent ant escape during feedings. Control A colonies were in standard ant boxes without Insect-a-Slip because I only thought to create this kind of control on the day of the manipulation and I did not have any extra Insect-a-Slip boxes prepared. I arbitrarily gave one group of pupae to each queen. I wanted the first clutch to consist only of the experimental pupae, so I removed the visible (post-first-instar) larvae on the day during which the pupae were dealt and on the following day. I removed all stages of larvae rather than just small larvae because workers produced during colony founding are notoriously small and so the relationship between larval size and coloration and maturity was hard to

judge. I removed larvae once the queens and their water tubes already had been placed in nests. To remove larvae from a nest, I removed the water tube, tapped the queen and brood out of the tube into the nest box. I then collected larvae from the nest box, from the inside walls of the tube, and from the cotton plug inside the tube. This process was inherently imprecise, because larvae are often embedded in the cotton, so some larvae may have been left in some colonies. I did not remove larvae from the unmanipulated control (Control A) colonies.

COLONY MAINTENANCE AND CENSUS

I maintained colonies in constant darkness at 30^{o}C. I methodically shuffled them within the incubator weekly to standardize the actual temperature experienced across colonies. Each week, the colonies were fed approximately 180 mg of a mixture of cracked wheat and crushed sunflower seeds, a small wad of honey-water-soaked cellulose, and a thawed previously-frozen fifth instar cricket.

I censused the adult workers in each colony weekly for the next sixteen weeks. I censused by eye and used a hand-counter starting on the sixth week post-manipulation (census 8/31/12), when the maximum colony size reached 27 workers. I mistakenly omitted fifteen colonies from the 8/17/12 census and one colony from the 8/3/12 census. I removed one data point corresponding to the 9/20/12 census for colony 19 from analyses because it was so high I suspect it was a typo. Data were entered directly into a computer spreadsheet, so I have no way of confirming this suspicion. I changed the 8/3/12 census of colony 362 from eight to one because it also was probably a typo; the colony had censuses of one

for the surrounding weeks and large numbers of carcasses were not observed. I performed all censuses myself.

ANALYSES

Three queens died during the experiment and their colonies were omitted from analyses. I also omitted from analyses four colonies whose growth during the experiment was negligible because such growth failure suggests poor quality of the queen and/or her mates, and I did not want individual variation in quality among the queens in this experiment. I also removed one extremely small queen (at least 20 mg smaller than the others) from the analysis. Thus 178 queens remained.

Though I intended the experimental first clutch sizes to be one, five, and ten workers, colonies did not all actually have the assigned number of adults at a standard time (e.g. three weeks post-manipulation) (Figure 3.3). It is possible that some pupae did not survive and that some larvae remained in the colonies through the manipulations and quickly made it to adulthood. I defined a colony's actual first clutch size as the number of adult workers present on the third week past the brood manipulation (i.e. its worker count on 8/10/12). The actual first clutches were concentrated around the intended first clutch sizes of one, five, and ten (Figure 3.4).

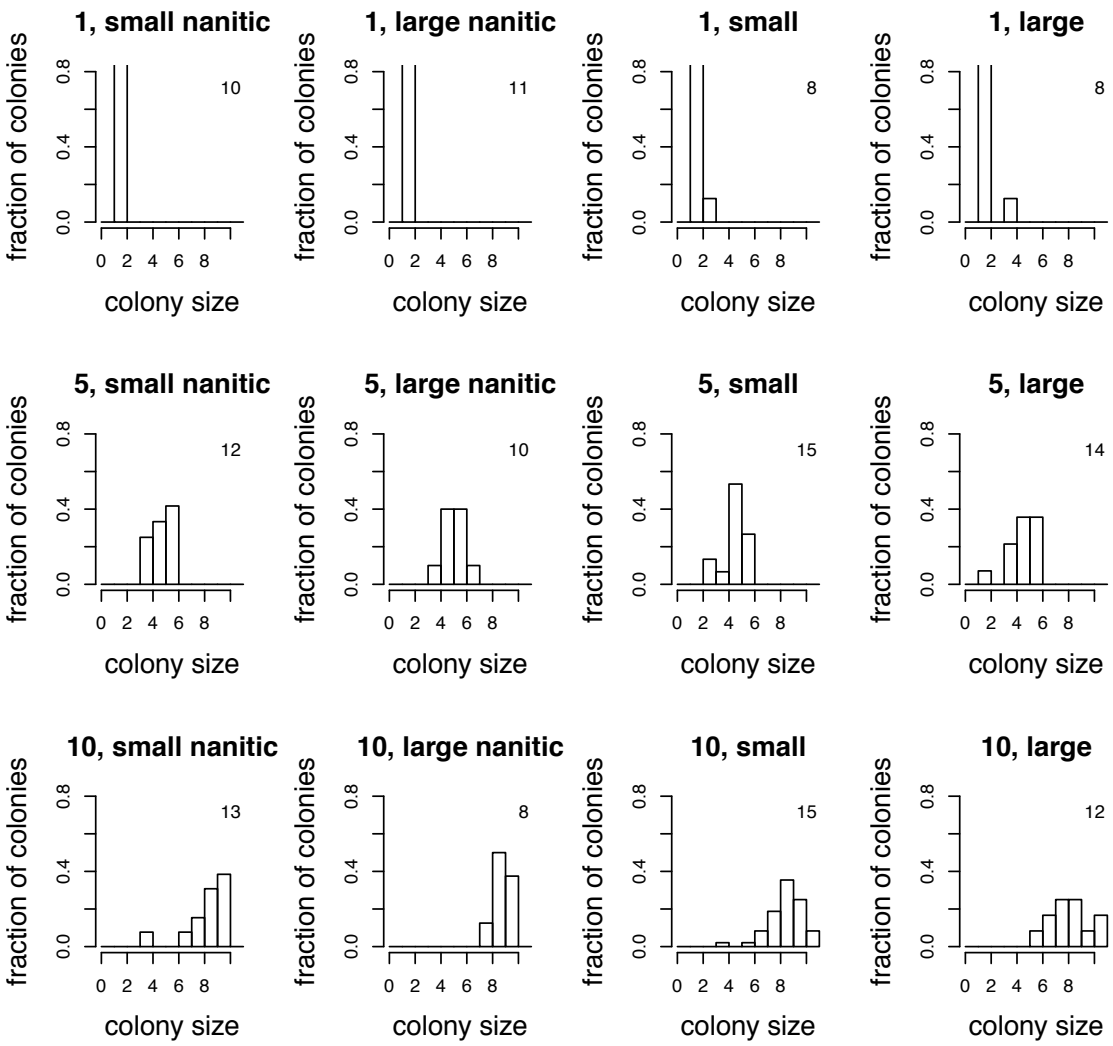

Figure 3.3: Intended vs. actual first clutch sizes. I defined first clutch size as the number of adult workers present in a colony at the third week post-manipulation. Plotted here for each intended experimental group are the actual colony sizes on day 21. For example, five of the colonies in the intended 1-worker, small nanitic treatment had zero adult workers. The numbers in the top right corners of each plot are the number of replicate colonies in that intended experimental treatment.

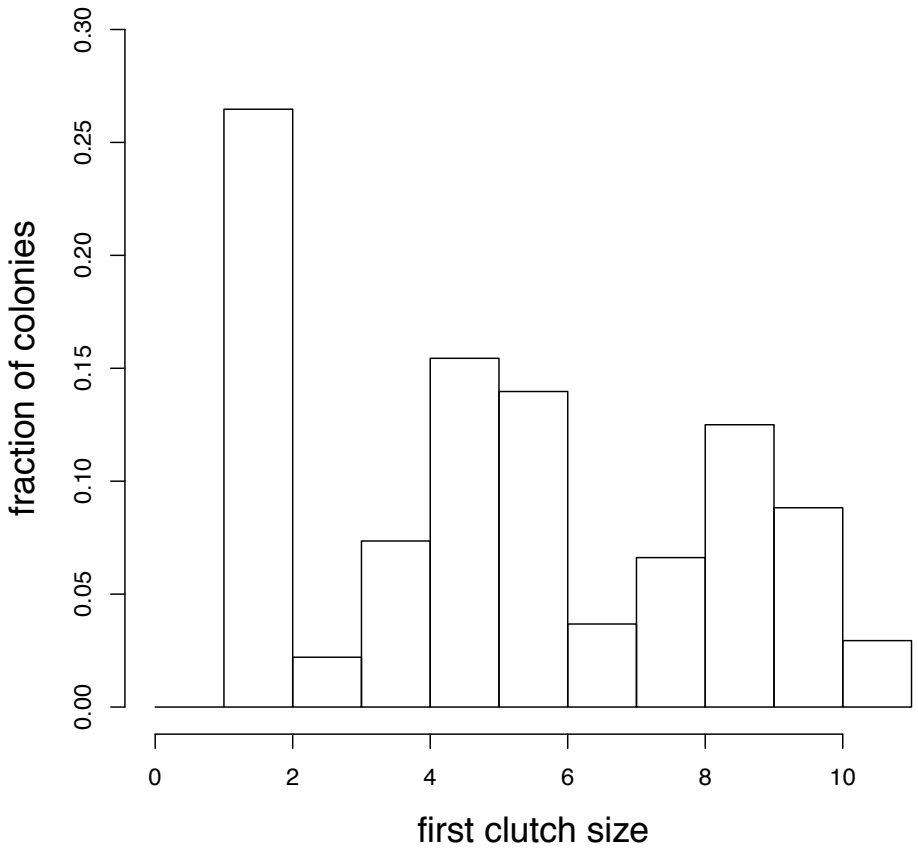

Figure 3.4: Actual first clutch sizes of experimental colonies. The number of adult workers present in each experimental colony three weeks post-manipulation was defined as the first clutch size.

For the analyses, the data were modified to represent colony growth beyond the first clutch. I took the actual first clutch size for each colony (census at week three post-manipulation) and subtracted it from the censuses of each of the following weeks. This yielded a count of the adult workers present in a colony not including the first clutch. I defined the day of the week three census (8/10/12) as day zero. I omitted from analyses all seventeen colonies which had a first clutch size of zero, i.e. which had zero workers on day zero. Those seventeen

colonies were all in intended 1-worker treatments, and their single worker presumably died. Thus 161 colonies remained.

Over the thirteen weeks (sixteen total weeks less three for the first clutch to eclose) of the experiment, colony growth did not follow a simple mathematical pattern; linear, exponential, and logistic models failed to capture the shape of growth when fit to the entire thirteen-week time course (weeks 3-16) (Figure 3.5).

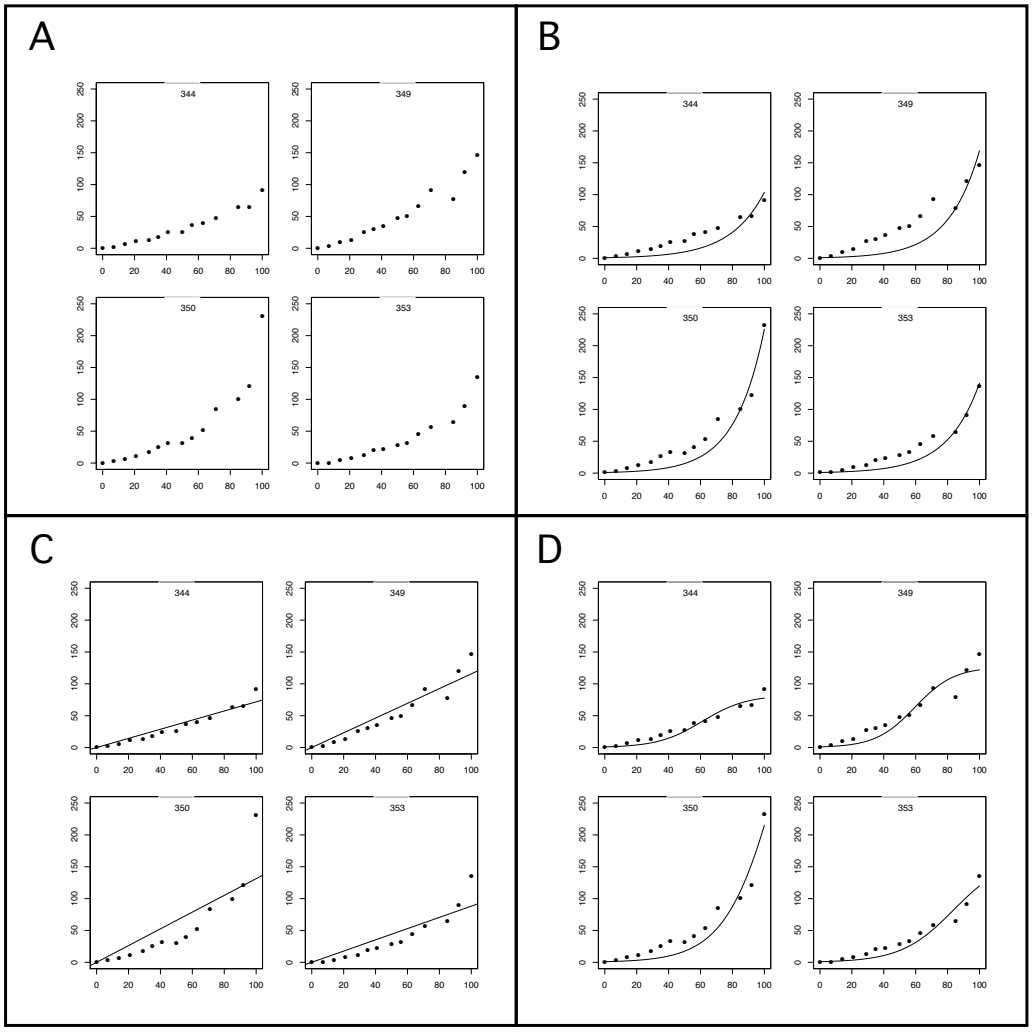

Figure 3.5: Growth of four sample colonies. x-values are the days since eclosion of the first clutch. y-values are the total number adult workers beyond the first clutch. In A are growth data for the four colonies without a model. The best-fit exponential, linear, and logistic models are plotted with the data in B, C, and D, respectively.

Linear models did, however, capture colony growth for the first nine weeks of data (weeks 3-11). I modeled each colony's growth during this time period with the following linear model:

$$N_t = rt$$

where t is day, r is the colony growth rate (in workers/day), and N_t is the colony size at time t. The y-intercept was fixed at zero for all colonies.

I used ANCOVA to determine the effects of initial worker size (headwidth), first clutch size, and queen initial wetmass on colony growth rate (r). Initial worker size was treated as an ordinal variable. I tested the directional hypothesis that greater initial worker size and greater first clutch size increase colony growth rate. Results were considered statistically significant at $P < 0.05$.

3.4 Results

I report the results for the 136 experimental colonies that had first clutches consisting of at least one worker. The workers composing the first clutch in the small nanitic, large nanitic, small non-nanitic, and large non-nanitic treatments had headwidths of 1.20 $\pm$ 0.06, 1.36 $\pm$ 0.07, 1.66 $\pm$0.09, and 2.06 $\pm$ 0.09 mm (mean $\pm$ sd, n = 223, 149, 241, 217 individual workers). First clutch size, defined as the number of adult workers present at the third week post-manipulation ("day zero") ranged from one to ten workers (Figure 3.4). Queen initial wetmass ranged from 54.7 to 82.5 mg with a mean of 67.2 mg (n = 136).

The linear fits to the first eight weeks of colony growth were good (R^2 = 0.97 $\pm$ 0.03, mean $\pm$ sd). I fit linear models to six, seven, eight, and nine weeks of

data and found that the fit generally improved with the inclusion of increasing weeks of data until week nine. I chose to analyze the first eight weeks because I wanted to include as much data as possible, and at nine weeks the linear fit starts to degrade (Figure 3.6) (i.e. the R^2 values are lower for linear models fit to nine-weeks of data than for linear models fit to eight weeks of data). Colony growth rate ranged from 0.16 to 0.92 workers/day with a mean of 0.54 workers/day (n = 136).

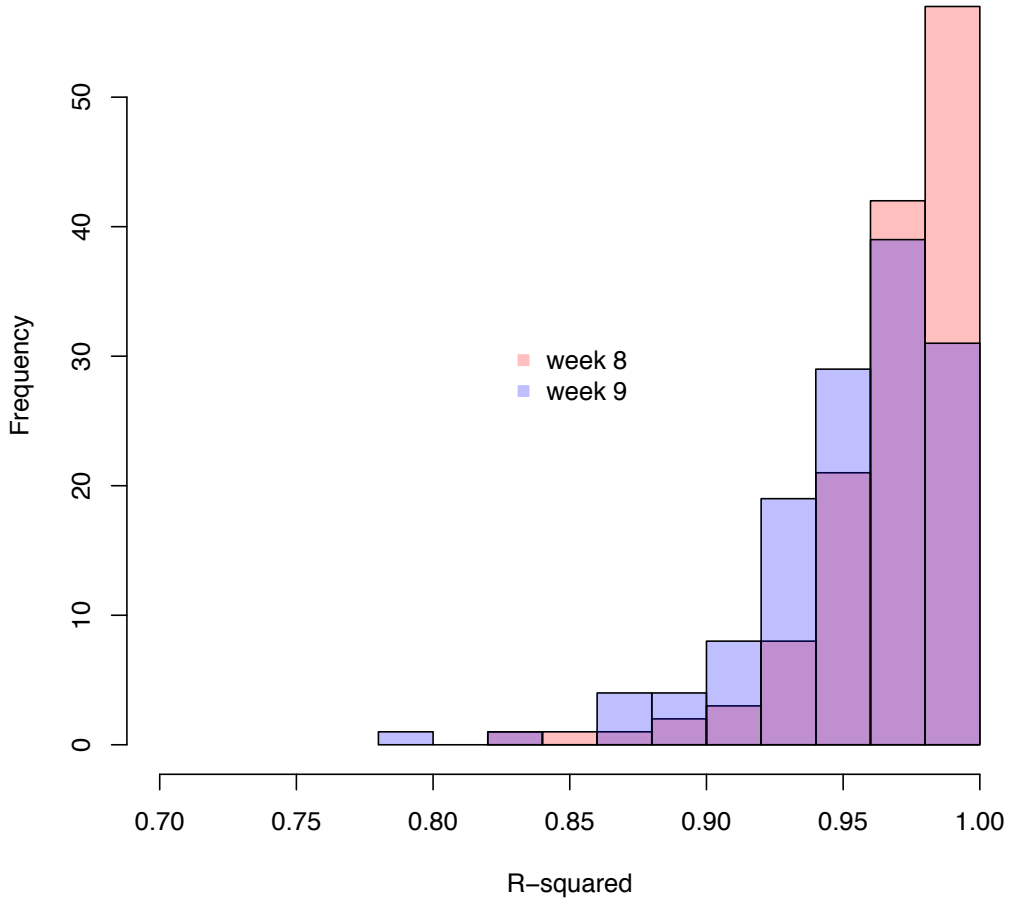

Figure 3.6: Comparison of model fits to eight weeks and nine weeks of the data

I fit a linear model to each colony's eight weeks of census data and used the slope as a measure of the colony's growth rate (*r*). I performed ANCOVA to evaluate the influences of queen initial mass, size of workers in the first clutch, and first clutch size on *r.* ANCOVA revealed an effect on *r* of initial clutch size ($P < 0.001$, $F_{1,127} = 37.4$) and queen initial mass ($P = 0.013$, $F_{1,127} = 6.4$) (Table 3.2).

Table 3.2: ANCOVA of first clutch size, worker size, and queen initial mass on *r*

Factor	DF	*F*	P
clutch size	1/127	37.4	< 0.001
worker size	3/127	1.2	0.315
queen initial mass	1/127	6.4	0.013
clutch size: worker size	3/127	1.0	0.413

To determine the direction of effect of queen initial mass on colony growth rate, I first removed the effects of first clutch size on colony growth rate by taking the residuals of the regression of first clutch size on colony growth rate. Then I regressed queen initial mass on these residuals. I used a similar approach to determine the direction of effect of first clutch size on colony growth rate. Colonies grew more quickly if they had larger queens or started with larger first clutches (Figure 3.7). The size of workers in the first clutch did not affect colony growth rate ($F_{3,127} = 1.2$, $P = 0.315$) (Figure 3.8).

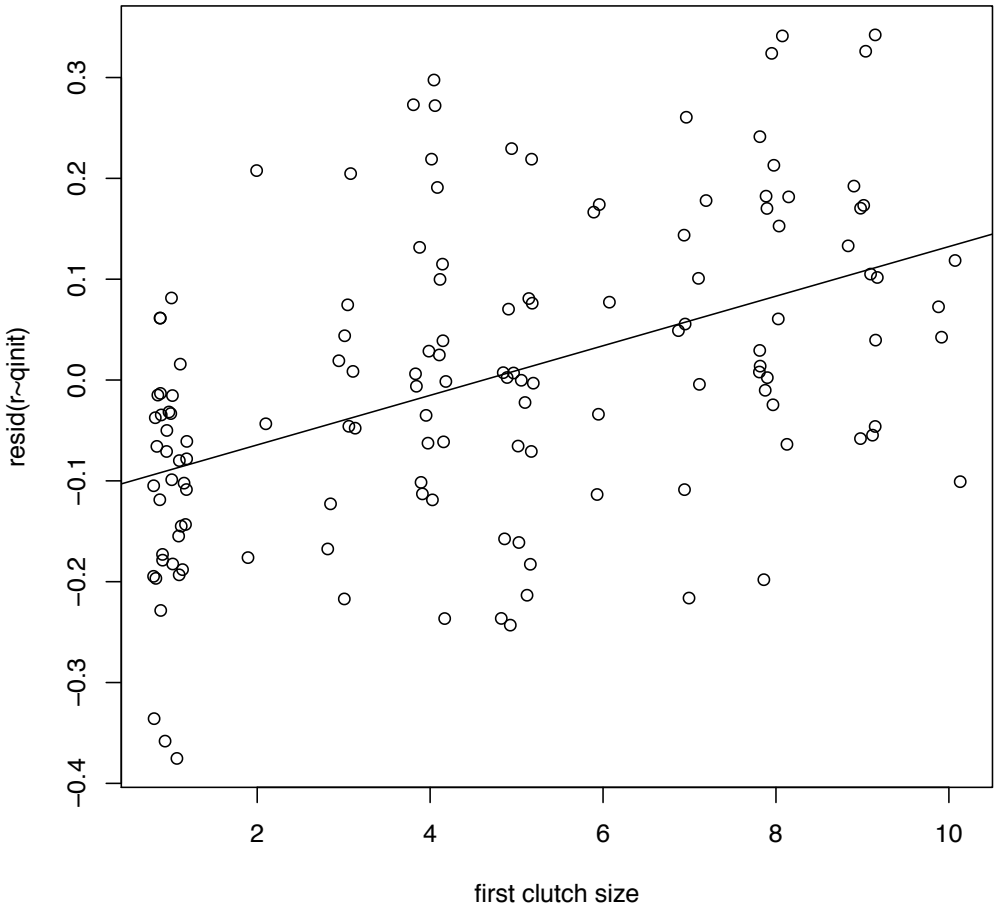

Figure 3.7: The effect of first clutch size on colony growth rate.

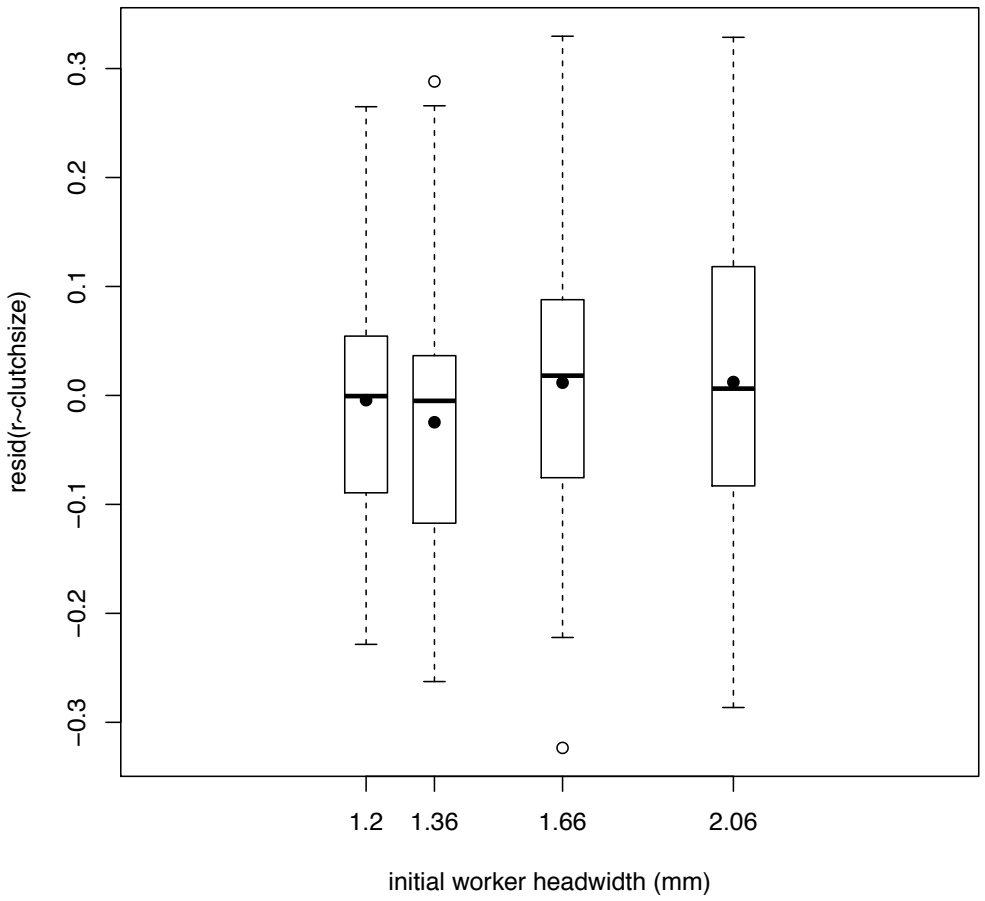

Figure 3.8: The effect of initial worker size on colony growth rate. Shown are medians (dark bars), means (filled circles), and first and third quartiles (tops and bottoms of boxes) of the residuals of colony growth rate on clutch size. Empty circles are points identified by R as potential outliers. Whiskers mark the minimum and maximum observations, excluding the outliers.

Clutch size manipulation has been shown to have deleterious effects on realized offspring number (Lack 1967), so I tested for an effect of clutch size manipulation on colony growth. I calculated the deviation of each queen's original first clutch size (the number of pupae she had before the manipulation) from the queen's actual first clutch size (the number of adult workers she had at week 3 post-manipulation) and regressed colony growth rate on the deviation in actual

from natural first clutch size. There was a significant positive relationship (R^2 = 0.11, $P < 0.001$): colony growth rate = 0.015*deviation + 0.55 (Figure 3.9).

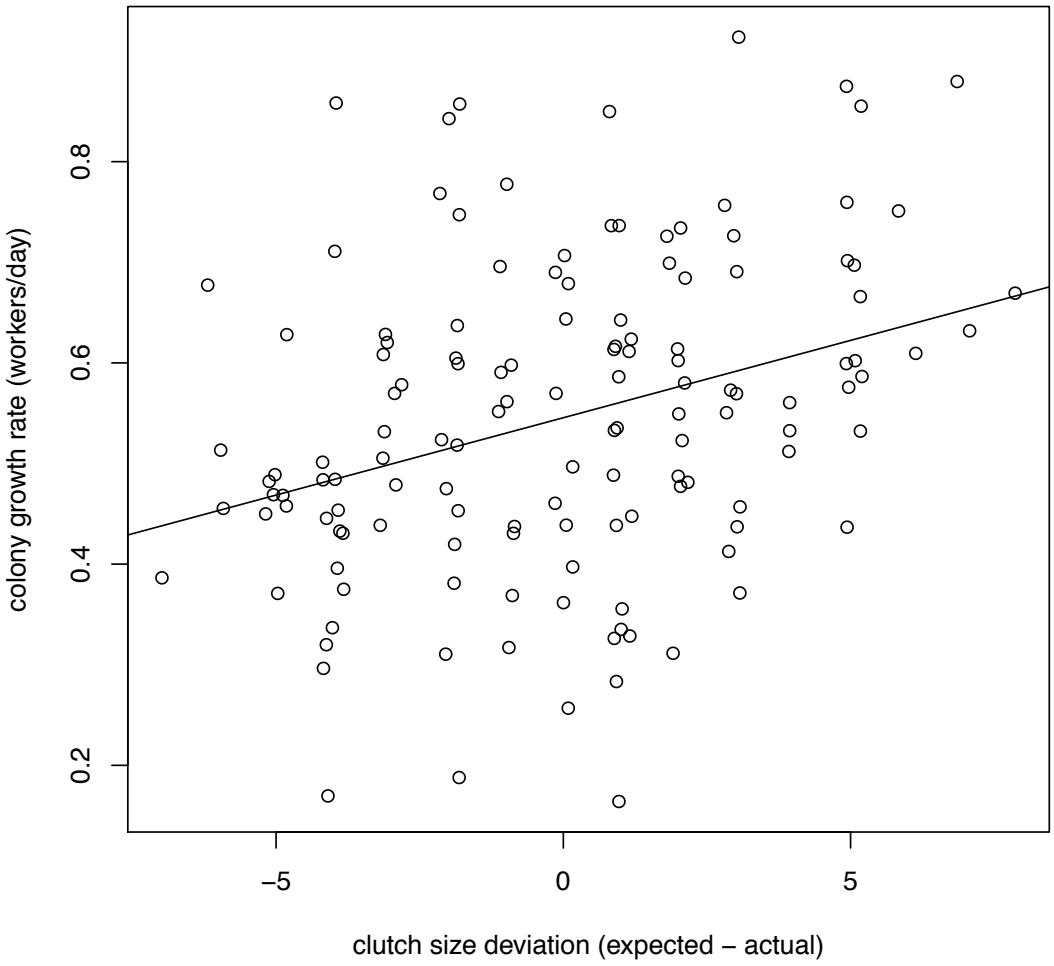

Figure 3.9: The effect of the deviation from natural clutch size on colony growth

To compare the performance of control vs. experimental colonies, I divided colonies into three groups based on first clutch size (Group 1: two or three workers, Group 2: four or five workers, Group 3: six or seven workers). Within each group, I pooled control colonies and did a Wilcoxon test for difference in colony growth rate from the experimental colonies within the same group. There was no difference in growth rate between control and experimental

colonies within each group (Group 1: W = 52, *P* = 0.516, Group 2: W = 157, *P* = 0.567, Group 3: W = 52, *P* = 0.516) (Figure 3.10).

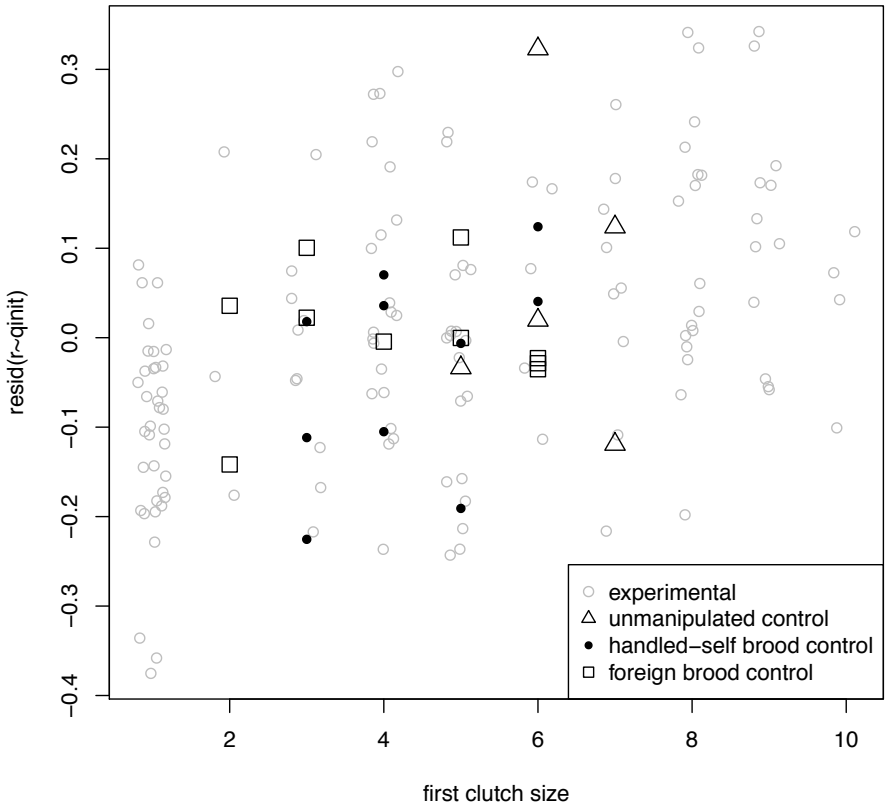

Figure 3.10: The effect of brood handling, brood substitution, and remnant first brood removal on colony growth. The growth rate of colonies in the control groups (Control A: unmanipulated, Control B: handled-self brood, and Control C: foreign brood) are overlaid on the growth rates for the experimental colonies.

3.5 Discussion

As in solitary species, in social insects, the winners during reproduction are those who leave more descendants. The main difference is that in solitary organisms, selection acts on individuals, and in social insects it acts on colonies. In social insects, then, the winners are the colonies that leave the most descendant colonies (Figure 3.12). At least one of the same factors that contributes to

descendant number in solitary organisms may also affect the success of a social insect colony; offspring size. Offspring size of solitary organisms is analogous to colony size, or the number of adult workers in a social insect colony. Just as a greater initial offspring size can accelerate offspring growth and enhance offspring survival in solitary organisms, colony first clutch size may accelerate colony growth and enhance new colony survival. Colonies with descendent colonies that are initially larger may ultimately leave more descendants.

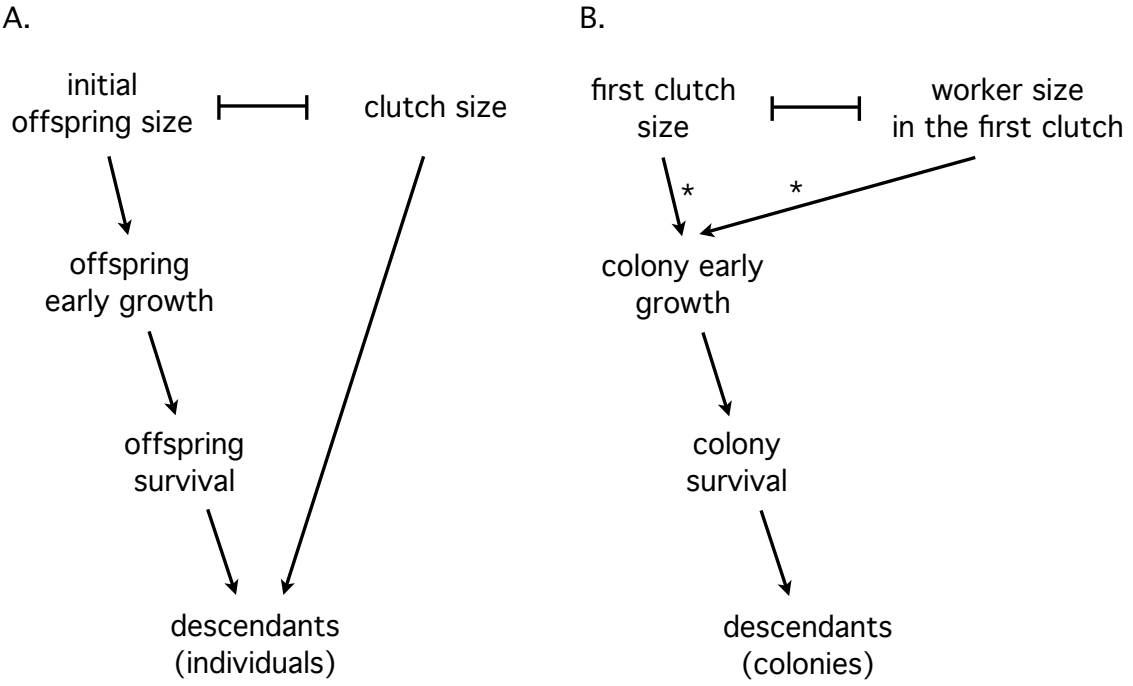

Figure 3.12: Putative relationship between offspring size and clutch size to fitness in solitary organisms and social insects. In solitary organisms (A), there is often a trade off between offspring size and clutch size, so though both traits can directly contribute to fitness, they can also indirectly contribute to fitness via inverse relations with each other. In social insect colonies (B), first clutch size is a good measure of initial offspring size because the final product of colony-level reproduction is a new colony. This trait and the size of workers in the first clutch may be determined in part by their relative influences on initial colony growth and therefore fitness. Asterisks (*) mark relationships measured in the present experiment.

This study suggests that in the queen's first individual reproductive allocation between worker number and worker size, worker number is more important. This finding is consistent with existing theory (Roff 2002). When juvenile mortality is high, as it is for social insect colonies, the optimal offspring size is high. The idea is that larger offspring can more quickly escape the dangerous juvenile stage than can smaller offspring. When, instead, adult mortality is high, organisms should produce as many offspring as possible, even if that means the offspring must be small. When survival is high in the juvenile vs. adult stage, increased investment in initial offspring size, or juvenile size, is likely to be of little benefit and may even reduce offspring survival.

The present results suggest that new queens that make larger first clutches (presumably of smaller offspring) in nature should grow faster. First clutch size may be even more important in the field than it was in the relatively benign conditions of this experiment. In nature, foragers are often lost to predation, so it could be important for field colonies to have large first clutches to buffer such worker loss. Worker number may also matter more in the field because more workers may mean more foragers, foraging success, and availability of resources for colony growth.

This study generated no evidence of direct consequences of worker size for growth of new colonies. The present study was limited in its assessment of the consequences of worker size. For example, this study could not detect a cost of the smallness of nanitics in terms of desiccation resistance because the ants were kept in humidified boxes. Worker size affects desiccation resistance

(Lighton et al. 1994; Hood & Tschinkel 1990), a key trait for species such as *P. barbatus*, some populations of which inhabit deserts. The relative importance of desiccation resistance for workers of incipient vs. mature colonies is unclear. Desiccation resistance may be more important to large colonies than to small colonies because foraging demand is determined in part by larval hunger, so new colonies (which all have few larvae) will have less of a need for the long foraging trips that involve great desiccation risk. On the other hand, larger colonies may command the foraging territories closest to them, and so workers in those colonies may not have to travel as far to find food.

Worker size may also affect foraging performance, with larger workers able to carry more food per foraging trip. A benefit of large worker size for foraging would imply a cost of small worker size for foraging, something that I could not measure in the present experiment because the ants were provided with *ad lib* food. Worker size affects foraging (Kaspari 1996). Foraging success is affected by forager number, which increases with colony size as measured by the nest cone, in *P. occidentalis* (Cole et al. 2008), but the relative effects of worker size and worker number on colony foraging performance have not been determined.

I may have failed to detect some true consequences of worker size because I assessed colony growth at a relatively low level of temporal resolution (weekly census). Real colony growth consists of individuals maturing from embryos into adults, and worker size and first clutch size may affect that rate of maturation. Though worker size did not affect colony growth rate in the present

study, worker size may affect development rate in *P. barbatus.* Worker size affects developmental time in fire ants, with nanitics developing more quickly than non-nanitics (Porter 1988). A simulation of the life history and population dynamics of fire ant colonies suggests that a small temporal advantage in eclosion of the first brood can make a big difference in long-term colony survival (Korzukhin & Porter 1994). Most of the variation in development rate among differently sized workers may occur before the pupal stage. If that is the case, then my experiment lacked this variation because I provided pupae of a standard stage across worker size treatments.

Other advantages of small size of nanitics have been proposed. Oster and Wilson (1978) state that survival of the first workers is key to colony survival (via the aforementioned task performance). The authors hypothesize that while foraging, workers are likely to encounter life-threatening predators, and that the smaller the worker, the less conspicuous it would be to these predators. The present study did not allow for a test of this hypothesis.

Consequences of body size *per se* for colony performance may become apparent with tests of workers of the entire range of sizes found in nature. *P. barbatus* workers collected from mature field colonies have headwidth of 2.5 mm, 25% larger than the large non-nanitics in the present study (Figure 3.13). Still, the current study involved workers spanning much of the full natural range of worker sizes, so the lack of a relationship between worker size and colony growth rate in the present study suggests that if there is a relationship between worker size and colony growth rate, it is non-linear.

The natural dynamics of worker size with colony size may reflect changing priorities in the allocation between worker size and clutch size. An independently-founded colony starts as a queen who produces a first clutch of workers which then produce more workers. As colonies grow in worker number, they produce larger and more variably sized workers (e.g. Brian 1957). Just as production of large first clutches helps colonies grow more quickly through the colony founding stage, production of larger workers by larger colonies may help those colonies maintain themselves during the later stages of colony life. Larger workers have lower metabolic rates, so they often live longer (Porter & Tschinkel 1985) and therefore contribute more to standing colony size (Asano & Cassill 2011). These lower metabolic rates also mean that colonies spend less energy to produce and maintain one gram of large workers vs. small workers (Porter & Tschinkel 1985). The results of the present study are consistent with the idea that new independently-founding social insect queens make small workers (nanitics) in order to make more workers to in turn accelerate colony growth. Beyond the founding stage, colonies may maintain their colony size most efficiently by producing larger workers.

The mechanisms behind the resource allocation pattern created by new queens in production of their first brood may be constant throughout the colony's life. Most organisms adjust their total reproduction (size and number of offspring) according to resource availability. In some cases, this adjustment may be a scaling up or down of the same general allocation pattern; the solution to the reproductive trade-off, or resource rationing among offspring size and number

may not change. As most colonies grow, they gain reproductive resources, and so, the widespread increase in worker size with colony size in nature may result simply from a scaling up of the same reproductive pattern that led to production of nanitics. Detailed quantification of clutch size and worker size variation across colony size may settle this issue. Also, new queens may be constrained in the size of offspring that they rear, independent of the fitness trade off with clutch size. New queens are physiologically different than older queens, and effects of queen age on worker size have been detected (independent of variation in colony size) in *Solenopsis invicta* (Wood & Tschinkel 1981). Because their ovaries are so recently maturing, new queens could be especially limited in the quality of eggs they produce. In abalone, egg quality increases from the first to the second clutches, in parallel with ovary maturation (Fukazawa et al. 2007). The second clutch is produced by abalone later in the season, when the ovaries of many individuals are more mature. The extent to which first clutch egg quality is proximately limited by ovary development *per se* in ants is unknown.

Also, the fact that the queen is alone in rearing the first batch of offspring has been found to limit the size and number of workers produced in the first clutch in *Pogonomyrmex occidentalis* (Chapter 2). Investigation of the mechanisms of offspring rearing and development in social insect colonies could clarify our thinking about reproductive patterns (worker size and clutch size) across the stages of colony life.

This study does not expose the mechanism of first clutch size contribution to colony growth rate. First clutch size may positively impact colony growth at two

stages of worker development: production of the fertilized eggs and survival of the eggs to adulthood. The simplest way to distinguish between these possibilities would be to (1) measure and compare the egg-laying rate in colonies with differently sized first clutches but similarly-sized queens and (2) assess the survival rates of set numbers of eggs given to colonies of various sizes. If first clutch size has its main effect on colony growth rate by increasing the rate at which the queen lays embryonated eggs, these eggs should appear at higher rates in colonies with larger first clutches. If first clutch size affects colony growth mainly by helping more embryos survive to adulthood, then embryos should survive at higher rates in larger colonies.

Acknowledgements
Diane Wiernasz contributed significantly to the design and analysis of this experiment and the development of the ideas in this chapter. This work was made possible by a grant from NHARP to D.W. Queen collection was successful because of Jessica Tarrand and Yen Saw. David Alvarado, Lulian Ho, and Stephanie Rice cared for the ants during the experiment. The graduate students in UH's Spring 2012 Population Biology seminar at the University of Houston suggested the manipulation of initial worker size in addition to number. Tony Frankino generated the idea to measure pupal headwidth using a scanner and lent a scanner for this purpose. Blaine Cole and Nat Holland helped with the analyses.

4 Determinants of brood care performance in the red harvester ant, *Pogonomyrmex barbatus*

Note: This experiment was executed jointly by myself and Stephanie Rice. Stephanie also contributed to the interpretation of the results.

4.1 Abstract

In many indeterminately growing organisms, larger adults produce larger offspring. This pattern also holds for social insect colonies as colonies grow in the number of workers they contain, they generally produce larger workers. The consequences of worker size for colony performance are unclear. I hypothesized that low mean adult worker body size confers a cost to colonies in terms of brood care. I created colonies of the red harvester ant, *Pogonomyrmex barbatus*, each consisting of adult workers of a narrow size range. Colonies were constructed such that mean adult worker size varied between colonies. Colonies received a standard amount of larvae, and I assessed the effects of adult worker size on the quality and rate of production of new workers. I performed two experiments that differed in multiple conditions, including the adult:brood ratio and food availability. Results varied between the two experiments. The results from one experiment suggest a small reduction of efficiency of small vs. large workers in brood care performance. These results are consistent with the idea that the natural pattern of worker size variation across colony development, with established colonies producing larger workers, is adaptive.

4.2 Introduction

Body size can affect organism performance. In insects, body size affects some traits directly, such as fecundity, metabolic rate, thermal resistance, venom content, and carrying load size (Calabi & Porter 1989; Honek 1993; Willott et al. 2000; Haight & Tschinkel 2003; Brown et al. 2004; Clemencet et al. 2010). These physiological traits may in turn affect other species-specific traits like defense performance and reproductive success (Kaspi et al. 2000; Haight 2010).

In social insects, worker body size can affect colony performance. Workers are the most abundant type of individual in social insect colonies, and they contribute to the colony fitness through performance of various tasks, including maintenance of the physical structure of the nest, defense of the inhabitants of the nest from predation, collection of nutrients for reproduction, and the rearing of the queen's offspring (their siblings). Workers can vary in size among species, among colonies within species, and even within a colony over time, and some consequences of worker size for colony performance are known. For example, Billick & Carter (2007) found that the high variance in worker size in *Formica obscuripes* colony fragments helped those colony fragments maintain their biomass over the course of three weeks of a limited diet.

Adult worker body size can affect the performance of brood care, a defining aspect of social insect colonies. When adult workers are present, *Solenopsis invicta* queens spend less than 1% of their time caring for brood (Cassill 2002). Siblings of the brood, adult workers, do most of the brood care, licking, feeding, and carrying larvae. The size of adult workers may affect their

brood care performance. Billick (2002) found that removal of large workers from *Formica neorufibarbis* colonies decreased the rates of new worker production. These production rates were lower than those of colonies naturally consisting of workers of a lower mean size, suggesting that variance in worker size is important for brood care. In *S. invicta,* however, variance in worker size did not affect brood care (Wood & Tschinkel 1981), but mean worker size did (Porter & Tschinkel 1985). The worker size composition of colonies in the Porter and Tschinkel experiment were confounded with colony size, but their results suggest that colonies composed of only large *S. invicta* workers are bad at brood care (Porter & Tschinkel 1985). Another caveat to that study is that natural *S. invicta* colonies are composed of a mixture of worker subcastes (majors and minors), so all-major colonies like those tested in that study are unlikely to be found in nature. Wood and Tschinkel (1981) found that brood production rates were the same for colonies with a high vs. low mean worker size, but in this experiment, mean worker size was positively correlated with variance in worker size.

Worker size may affect brood care in other species. In seed harvester ants, workers mill seeds (Wiernasz, personal observation). Adult workers contain high levels of amylases (Wiernasz, unpublished data) and may use these to help larvae digest the starch of seeds. Larger workers have larger jaws (Tschinkel et al. 2003), so larger workers may be better at preparing food for larvae. On the other hand, smaller workers have higher metabolic rates and so may have higher rates of activity and therefore may tend to larvae more frequently (Porter & Tschinkel 1985).

Worker size changes naturally with colony development. An increase in mean worker size with colony size is a common pattern among social insect species whose colonies are founded independently, without adult workers (reviewed in Wood & Tschinkel 1981). In *Pogonomyrmex occidenalis*, for example, workers collected from one-year-old field colonies are on average 34% smaller in headwidth than workers collected from mature field colonies, that are probably more than 15 years old (Cole & Wiernasz, unpublished). The first workers (nanitics) produced by incipient colonies are extremely small.

Little is known about the colony performance consequences of these natural changes in worker size with colony size. I focus on the consequences of the small size of nanitics. Nanitics' small size may affect colony performance indirectly, allowing a queen to produce a larger first clutch, which accelerates early colony growth (Chapter 3). High growth rate may be particularly important during colony founding for species whose colonies are founded independently. However, there is also some evidence that nanitics are costly, in terms of brood care performance (Porter & Tschinkel 1985). Porter & Tschinkel (1986) found that new *S. invicta* queens provided with adult non-nantics produced larvae and pupae at a higher rate than did queens who had an equal number of nanitics. I considered worker size as a continuous variable, rather than nanitics vs. non-nanitics because worker size varies continuously in my study species. Here I test the hypothesis that low mean adult worker size is costly in terms of brood care performance in the red harvester ant, *Pogonomyrmex barbatus. I* expected costs of small workers to appear in this experiment as decreased brood development

rates, brood survival probabilities, and/or new worker sizes for colonies composed of smaller (vs. larger) workers. If, instead, small worker colonies rear brood at equal or higher rates as large worker colonies, the alternative hypothesis that the low mean adult worker size found in young colonies is inconsequential for brood care would be supported.

4.3 Materials and Methods

POGONOMYRMEX BARBATUS

Pogonomyrmex barbatus are relatively large red ants common throughout the southwestern United States and Mexico. My source population is in Katy, Texas. Mean worker headwidth in established field colonies ranges from 2.11-2.40 mm, (n = 10 colonies, 12 workers measured per colony, workers collected in 2012 at Bear Creek Park in Katy, Texas) (Table 4.1). Queens of this species found colonies independently (without adult workers), individually (without additional queens), and claustrally (without foraging). Colonies grow for the first few years via production of workers and then may reproduce annually for the rest of their lives. Colony reproduction consists of production of winged males and females that aggregate from multiple colonies at a few common sites and mate (McCook 1879). Mated females then disperse and dig new nests.

Table 4.1: Adult worker size in ten field *P. barbatus* colonies

mean	sd
2.4	0.0816
2.19	0.1327
2.15	0.0779
2.21	0.095
2.24	0.0752
2.17	0.0639
2.23	0.099
2.28	0.095
2.11	0.0912
2.17	0.0656

n = 12 workers per colony

To test the hypothesis that nanitics are costly to colonies in terms of brood care performance, I performed two experiments. I ran the first experiment soon after the June 2012 mating flight. Preliminary analyses suggested a negative result (no effect of worker size on brood care performance) which I suspected arose because in the conditions of the first experiment workers of all sizes could rear brood so well that true differences in the brood care abilities of differently sized workers were not detectable. To test this idea, I ran a second experiment in December 2012/January 2013 with a lower adult:brood ratio and lower food availability.

EXPERIMENT 1

COLONY CONSTRUCTION

Fifty-one queens were collected within a day of their mating flight in June 2011, in Bear Creek Park in Katy, Texas, and used to construct the colonies for this experiment. The queens were allowed to found their colonies in nests consisting of clear plastic boxes (17 x 12 x 6 cm) containing a test tube partially filled with water and stoppered with a cotton ball. A year later the queens were removed from their colonies and placed individually in new nests without workers. These queens were part of the constructed colonies. I put a queen in each constructed colony because *Pogonomyrmex* workers can have different brood care patterns with vs. without a queen (e.g. rearing males in the absence of a queen) (personal observation). To these queens, I added workers in the form of pupae that were gathered from approximately 100 one-year-old lab stock colonies. Some colonies that were used as a source for queens were also used as a source for workers, so it is possible that some constructed colonies contained some workers who were actual offspring of their “new” queen. To make pupal collection easier, stock colonies were chilled at 4^{o}C for up to an hour (because it takes a while for the insides of the plastic nest boxes to change temperature). Pupae were then collected using fine forceps. To limit the variance in worker age within constructed colonies, I chose pupae that were white (i.e. early in pupal development) but had pigmented eyes (i.e. not the earliest stage of pupal development which is white without pigmented eyes).

Before putting the pupae in the new colonies, I grouped them into sets of approximately 50, roughly according to size and measured them. Pupae were first pooled and manually sorted according to size by the unaided eye. Then, to precisely quantify worker size, I scanned the ventral side of each pupal group on an Epson Perfection V500 Photo Scanner and used Image J to measure the headwidth, or distance from the outside edge of one eye to the other, to the nearest 0.01 mm. I then placed each group of pupae into a new nest. For each of the constructed (experimental and self-pupa control) colonies, I calculated the mean headwidth of pupae used to construct it. I call this variable adult worker size. To help the pupae eclose, I temporarily kept five of the queen's original adult workers in the new nest. The five adult workers were removed within a week of colony assembly, when the pupae had begun to eclose.

Some of the 50 pupae that were placed into each nest did not eclose, resulting in colony size variation among colonies, from 28 to 45 workers, with a mean of 36. These counts were derived from a census taken of each constructed colony immediately before the experiment (i.e. before adding larvae for rearing), and I used this colony size as a covariate in the brood care performance analyses.

Because the supply of pupae from lab stock colonies was limited, I constructed colonies (and then ran the experiment) in five temporally distinct blocks. Each colony was run in the experiment only after all its pupae had eclosed. This means that each colony was run from one to three weeks from the time of its own construction.

Three of the 51 colonies in this experiment were constructed using nanitic pupae pooled from the first clutches of queens collected in the 2012 mating flight. To make these three colonies, 50 nanitic pupae were given to each queen.

I also made eighteen control colonies. Half of these, self-pupae control colonies, consisted of a queen from the 2011 mating flight and the pupae present in her colony one year later. The other nine "adult" control colonies were made with adult workers and queens from 2011. For each queen, I arbitrarily chose 40 of the adult workers present in her colony one year later.

COLONY MAINTENANCE

Colonies were maintained in constant darkness in a 30^{o}C incubator and fed weekly honey-water soaked cellulose, a mixture of crushed sunflower seeds and cracked wheat, and a thawed (previously-frozen) 5th instar cricket. Colonies were fed additional crickets as needed, approximately every other day, to ensure food abundance. Crickets were provided in abundance because insect protein is the essence of a high quality diet for harvester ants (Smith and Suarez 2010).

BROOD CARE PERFORMANCE

I tried to standardize the brood care challenge across colonies by providing the colonies with larvae to rear. In this experiment, I used larvae from a range of developmental stages. A *Pogonomyrmex barbatus* ant larva is white when it hatches from its egg. As the larva develops, it turns yellow and then brown/black, and finally white again just before pupation (Figure 4.1). The smallest larvae I

used were small and white (stage A in Figure 4.1). These larvae are the smallest larvae whose survival is not strongly affected by manipulation. The largest larvae I used in this experiment were of stage C. I collected the larvae (stages A-C) from one-year-old lab stock colonies, and then distributed the larvae arbitrarily into groups of fifteen, regardless of larval stage. I then weighed each group to the nearest 0.01 mg on an analytical balance (AT20 Mettler Toledo, Ohio, USA), and arbitrarily dealt one group to each experimental colony. An adults:brood ratio higher than 1:1 is common in ant colonies in nature (Lavigne 1969).

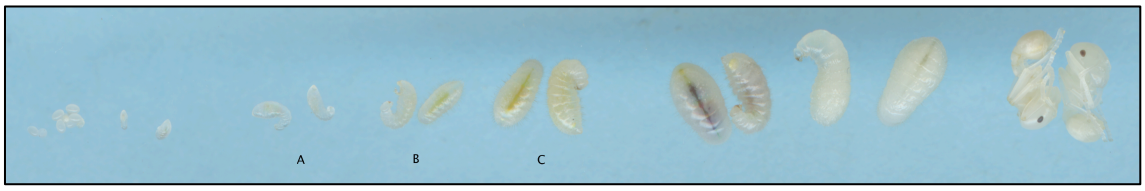

Figure 4.1: Stages of *Pogonomyrmex barbatus* worker development. Workers develop from eggs to larvae to pupae, before eclosing as adults. I added larvae to colonies constructed of adult workers and a queen. For Experiment 1, I used larval stages A-C. For Experiment 2, I used larval stages A and B.

The average per capita initial wetmass of larvae dealt to a colony was calculated as the total wetmass of the group of 15 larvae divided by 15. I called this larval size. Larval size varied across larval groups, 2.3 – 6.1 mg. I incorporated this unintended variation into the brood care performance analyses by including larval size as a covariate.

I counted the larvae in each colony daily for the first six days of the experiment. The larval counts for some colonies dropped by zero to three larvae early on, mainly during the first three days; of the fifteen larvae dealt to each colony, 13.5 $\pm$ 1.3 (mean $\pm$ s.d.) survived to day three. I think that this larval

death early in the experiment was due to human handling rather than brood care, and so I considered the number of larvae in each colony on day three as the colony's total starting number of larvae. I checked the colonies every few days for pupae. I noted the day at which each colony made its first pupa. When a colony had pupated nearly all of its original (dealt) larvae, I checked it every day until the last original larva pupated. I noted the day at which each colony made its last pupa.

Whenever a colony had a darkly colored pupa, I removed all of that colony's pupae. This ranged from one to eighteen pupae. Because I only gave fifteen larvae maximum to each colony, this means that some of the pupae collected were reared from eggs produced by the queen during the experiment. If I collected "extra" pupae for a colony, I looked at that colony's original total larval count and chose the appropriate number of pupae that were most lightly colored (indicating relative immaturity) to exclude from analyses.

I assess new worker size by imaging pupae with the scanner method described above.

I continued to monitor each colony until it had produced pupae equal in number to the total original larvae. For example, from a colony that had thirteen larvae on day three, I collected and measured thirteen pupae.

ANALYSES

To determine the effects of adult worker size, larval size, and colony size on colony brood care performance, I constructed linear models and performed

ANOVA (Table 1). Each model contained one dependent variable (time to first pupa, time to last pupa, mean new worker size, or variance in new worker size) and all three independent variables. In the model for new worker size, all individual new worker headwidth measurements were used (multiple measurements per colony), and the analysis took into account the lack of independence of headwidths of new workers from the same colony by nesting workers within colonies. I fit a linear mixed-effects regression model, regressing new worker size on the independent variables of mean adult size, larval size, and colony size (lme (new worker size ~ mean adult size + larval size + colony size, random = ~1|colonyID)). Results were considered statistically significant at $P < 0.05$. R was used for all analyses (R core development team).

EXPERIMENT 2

COLONY CONSTRUCTION

Eighteen queens were collected within a day of their mating flight in June 2011. Colonies were constructed from pupae and newly eclosed adults that had been kept individually in the wells of 96-well plates. These pupae were originally taken from ½-year-old colonies (reared by queens collected after a 2012 mating flight), and their headwidths were measured for another experiment using the photo-scanner method described for Experiment 1. I used the workers in the plates to construct colonies a couple of weeks later. By that time some individuals in the plates had eclosed and some had died. I used the precise worker measurements to group workers in the plates into sets of approximately 50-80 and chose the

individuals that were live adults or pupae that appeared viable. Approximately 40 workers (as adults or pupae) were used to make each colony.

To increase the sample size of Experiment 2, five additional colonies were made, but with a different method. I collected pupae directly from six-month-old lab colonies, measured them, and placed them with queens collected in 2011. Two of these colonies consisted of very large workers and the remaining three consisted of very small but non-nanitic workers. Larvae in all these five colonies developed more slowly than larvae in the other colonies in this experiment (average time to first pupa = 16.2 days for these 5 colonies vs. 12.4 days for the other colonies in this experiment). Because of the large difference in developmental time and the difference in age of the source queen, I decided to omit the data from these five colonies from the analyses.

COLONY MAINTENANCE

Colonies were provided with *ad lib* seeds and honey water and no crickets. Honey water was replaced weekly and seeds were added as needed. The incubator was set at 12:12 light:dark and maintained at 30^{o}C.

BROOD CARE PERFORMANCE

Larvae were harvested from six-month-old lab colonies. For this experiment I used only small white and small yellow larvae (stages A and B in Figure 1), earlier stages than the stages of some of the larvae used in Experiment 1. For each experimental colony I censused the adult workers and made a group of

larvae of the same size. For example, for a colony with 38 adult workers, I made a group of 38 larvae. I weighed these groups of larvae and calculated the mean per capita larval wetmass for each group. I shuffled larvae among groups and swapped them with stock larvae until the larval size for all colonies was 2.2 – 2.6 mg. I gave each colony its corresponding group of larvae. Because colonies were dealt the same number of larvae as there were adults in the colony, the adults:brood ratio in this experiment was 1:1.

In this experiment, I monitored the colonies for the first and last pupation. In Experiment 1, the minimum time to first pupation was nine days, so in Experiment 2 I monitored colonies only every few days until one week after the start of the experiment. I censused a colony daily upon appearance of its first pupa. I stopped taking data on some colonies on day 22 because at that time the brood in those colonies' nests consisted only of pupae (presumably from experimental larvae) and very small brood (presumably from eggs laid by the queen during the experiment). None of the experimental brood had eclosed by day 22. Though colonies were given 25-40 larvae, only six out of the eighteen total colonies in this experiment produced more than 20 larvae by day 22, suggesting that some larvae died during the experiment. To estimate larval survival, I regressed the number of pupae present at day 22 on the number of experimental larvae. I called the residuals of this relationship brood survival.

ANALYSES

To determine the effects of adult worker size, larval size, and colony size) on colony brood care performance, linear models were constructed and ANOVA performed as for Experiment 1 (Table 1). I made one model for each dependent variable. The dependent variables were time to first pupa, mean new worker size, and variance in new worker size. I also tested for an effect of the independent variables on brood survival rates. I took the arcsine-squareroot transform of the proportion of pupae surviving to day 22 and ran an ANOVA with those values at the dependent variable. In the model for new worker size, measurements of all individual new workers were used (multiple measurements per colony), and the analysis took into account the lack of independence of measurements of new workers from the same colony by nesting workers within colonies. I used the following linear mixed-effects regression model: lme (new worker size ~ adult worker size + larval size + colony size, random = ~1|colonyID). Results were considered statistically significant at $P < 0.05$. To compare brood care performance between experiments, I used a Wilcoxon Rank Sum test.

4. 4 Results

EXPERIMENT 1

The 51 colonies in Experiment 1 consisted of 36 $\pm$ 5 adult workers (mean $\pm$ s.d.). Pupal mortality was non-randomly distributed among colonies ($P = 0.017$, $R^2 = 0.11$, pupal loss = 6 * worker size + 2.56) (Figure 4.2). In other words, colonies composed of smaller adult workers had more adult workers at the start of the

experiment. Among colonies, mean adult worker headwidth ranged from 1.19 to 2.32 mm. The standard deviation in adult worker headwidth within a colony was, on average, 0.09 mm.

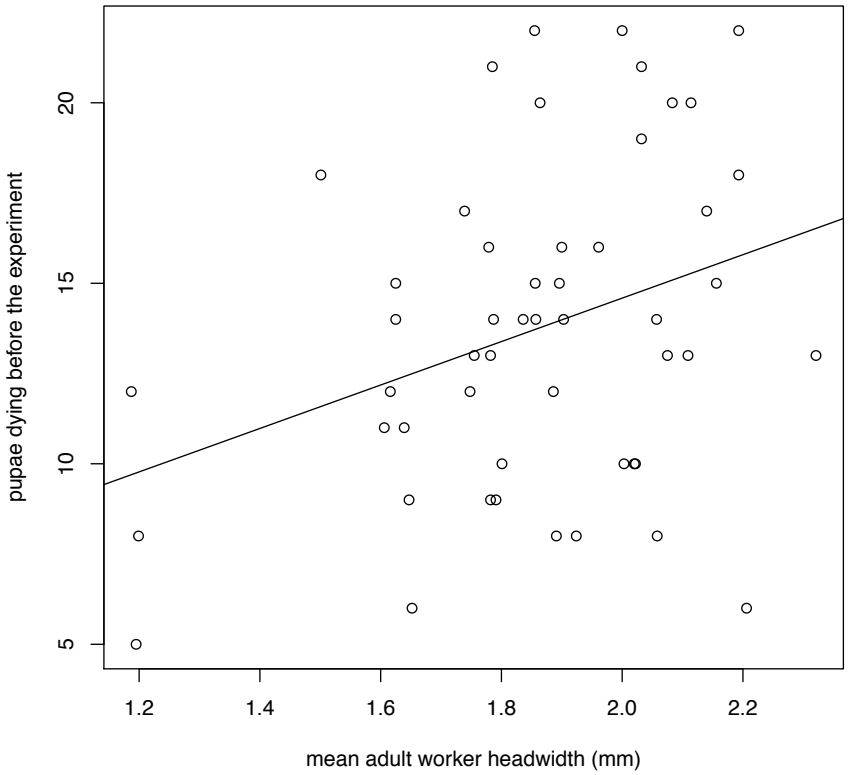

Figure 4.2: Mortality of pupae in constructed colonies in Experiment 1. There is one point for each experimental constructed colony.

Table 4.2: ANOVA results for Experiment 1

Factor	DF	*F*	P
Time to first pupa			
adult worker size	1/47	1.7	0.200
colony size	1/47	6.7	0.013
larval size	1/47	19.0	<0.001
Time to last pupa			
adult worker size	1/47	0.6	0.437
colony size	1/47	2.1	0.159
larval size	1/47	0.2	0.696
New worker size			
adult worker size	1/47	10.8	0.002
colony size	1/47	4.3	0.045
larval size	1/47	6.1	0.018
Variance in new worker size			
adult worker size	1/47	1.7	0.200
colony size	1/47	0.0	0.975
larval size	1/47	0.3	0.580

In the model for time to first pupa, two of the three independent factors included in the model were significant: colony size and larval size (Table 4.2). Brood matured more quickly in colonies with fewer workers (Figure 4.3). Brood matured more quickly from initially larger larvae (Figure 4.4). A trend towards a negative relationship between adult worker size and time to first pupa was non-significant.

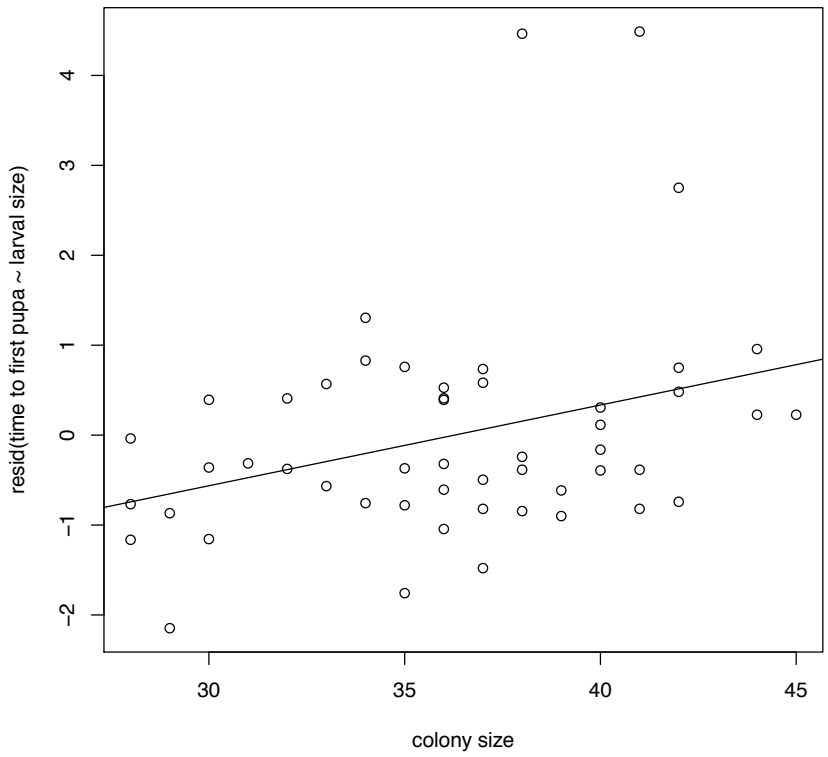

Figure 4.3: Effects of colony size on new worker development rate

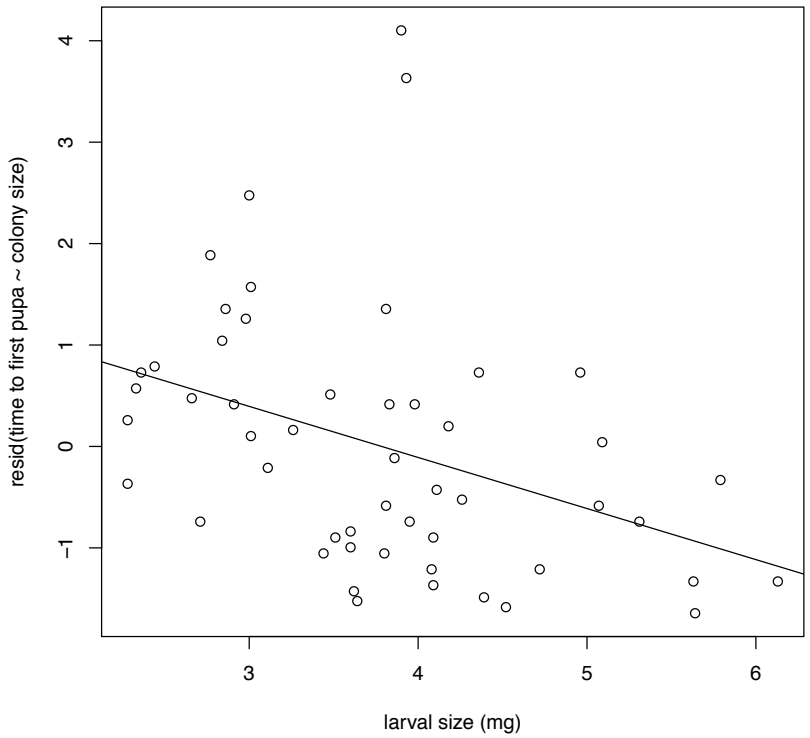

Figure 4.4: Effects of larval size on new worker development rate.

All of the independent variables, adult worker size (P = 0.002, $F_{1,47}$ = 10.8), colony size (P = 0.045, $F_{1,47}$ = 4.3), and larval size (P = 0.018, $F_{1,47}$ = 6.1), affected new worker size (Table 4.2). Colonies consisting of more adult workers produced larger workers, and colonies starting with larger larvae produced smaller workers. Colonies consisting of larger adult workers produced larger workers (Figure 4.5). When the nanitic colonies were dropped from the analysis, the effect of adult worker size on new worker size dropped below significance, but the trend remained ($F_{1,44}$ = 3.77, P = 0.059). Dropping nanitic colonies from the analysis did not remove the effects of larval size and colony size on new worker size ($F_{1,44}$ = 6.26, P = 0.016 and $F_{1,44}$ = 4.58, P = 0.038, respectively).

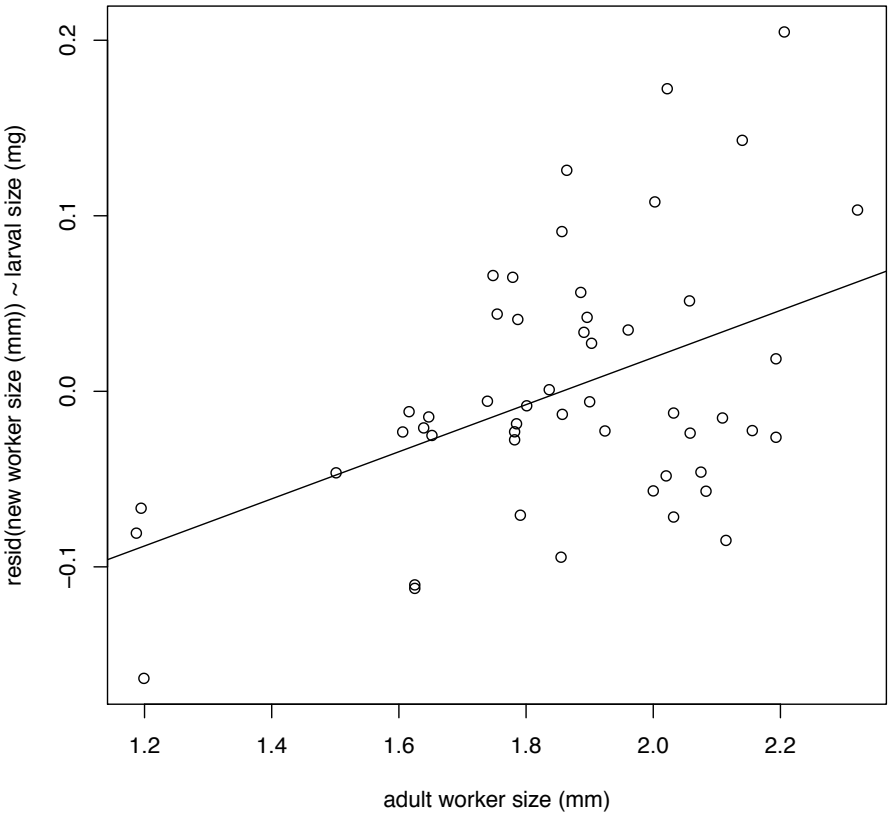

Figure 4.5: Effects of adult worker size on new worker size.

Adult worker size, larval size, and colony size did not affect the day of pupation of the last experimental brood item (Table 4.2). There was no relationship between mean new worker size and time to first pupa (R^2 = 0.02, df = 1, 49, *P* = 0.371). Variance in new worker size was not affected by any of the independent variables tested.

EXPERIMENT 2

The 18 colonies in this experiment consisted of 35 ± 5 workers and 31 ± 5 (mean ± s.d.) at the beginning and end of the experiment, respectively. Among colonies, mean adult worker headwidth ranged from 1.47 to 2.29 mm. Adult worker size varied little within colonies (on average, s.d. = 0.03 mm).

Table 4.2: ANOVA results for Experiment 2

Factor	DF	*F*	P
Time to first pupa			
adult worker size	1/14	6.4	0.024
colony size	1/14	0.8	0.374
larval size	1/14	0.5	0.485
arcsin(brood survival)			
adult worker size	1/14	3.9	0.069
colony size	1/14	0.5	0.493
larval size	1/14	0.6	0.439
New worker size			
adult worker size	1/14	0.1	0.741
colony size	1/14	0.1	0.828
larval size	1/14	0.0	0.900
Variance in new worker size			
adult worker size	1/14	0.1	0.758
colony size	1/14	0.3	0.567
larval size	1/14	0.4	0.529

Adult worker size affected time to first pupa (P = 0.024, $F_{1,14}$ = 6.4) (Table 4.2). Colonies consisting of larger adults produced their first pupae later than did colonies consisting of smaller adults (Figure 4.6). An increase in adult worker headwidth of 0.1 mm decelerated first pupation by approximately half a day. Adult worker size did not affect new worker size (P = 0.741, $F_{1,14}$ = 0.1) (Figure 4.7). A trend toward lower brood survival for larger adult workers was not significant.

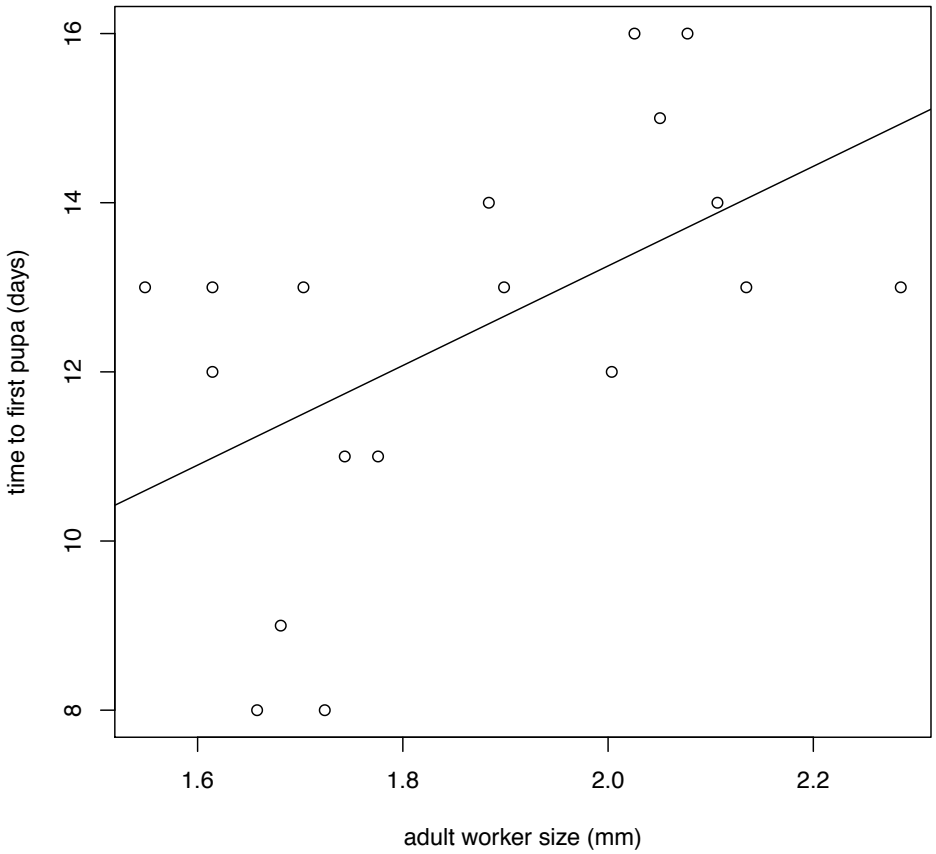

Figure 4.6: Effect of adult worker size on time to first pupa.

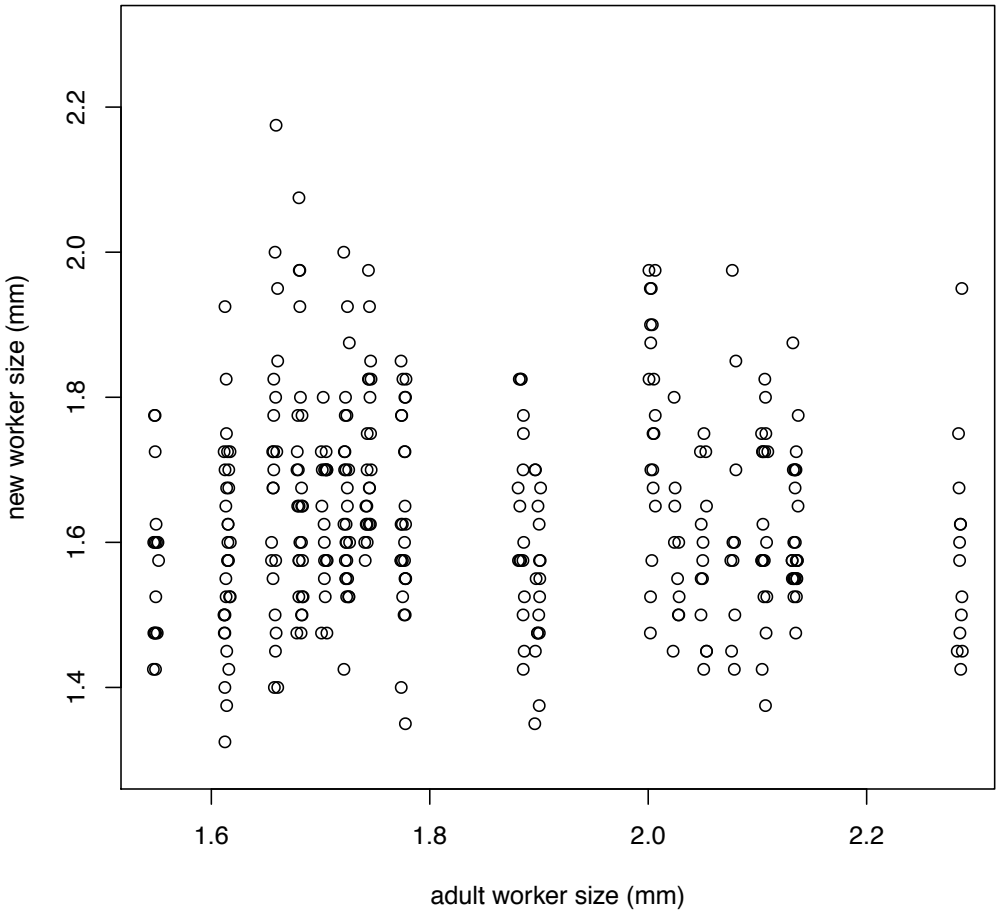

Figure 4.7: Effect of adult worker size on new worker size. Each point represents an individual pupa produced by a colony with the displayed adult worker size.

COMPARISION

Workers produced in Experiment 2 were smaller than those produced in Experiment 1 (W = 897, $P < 0.001$) (Figure 4.8). Mean new worker size differed between the two experiments by 0.18 mm, which corresponds to approximately a 10% decrease from Experiment 1 to Experiment 2.

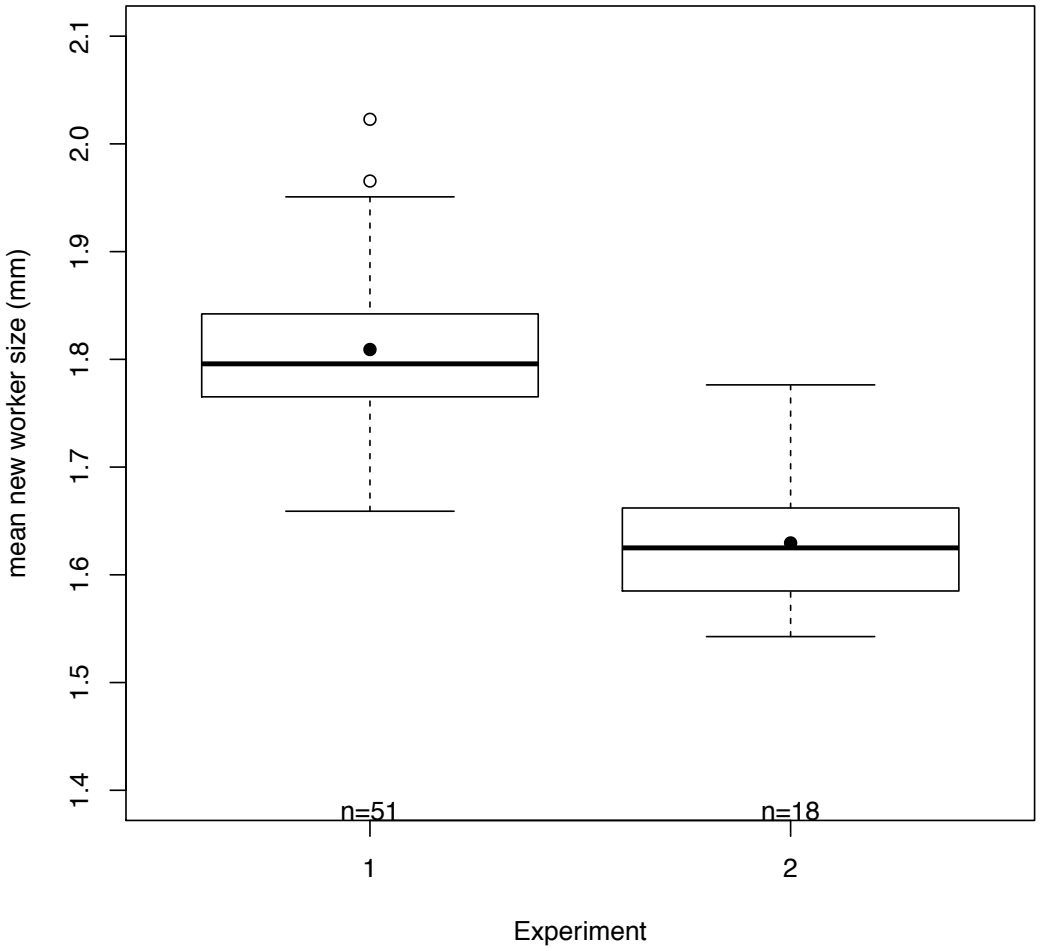

Figure 4.8: Effect of experimental conditions on new worker size. The medians (dark bars) and quartiles (tops and bottoms of boxes) of size of worker produced in Experiment 1 and Experiment 2. Filled circles represent the mean size of worker produced in each experiment. Empty circles are points identified by R as potential outliers. Whiskers mark the minimum and maximum observations, excluding the outliers.

Time to the first pupa was shorter in Experiment 1 than in Experiment 2 (W = 302.5, *P* = 0.029) (Figure 4.9). The mean day of first pupation was approximately one day earlier in Experiment 1 than in Experiment 2. This corresponds to an approximately 7% deceleration in first pupation from Experiment 1 to Experiment 2.

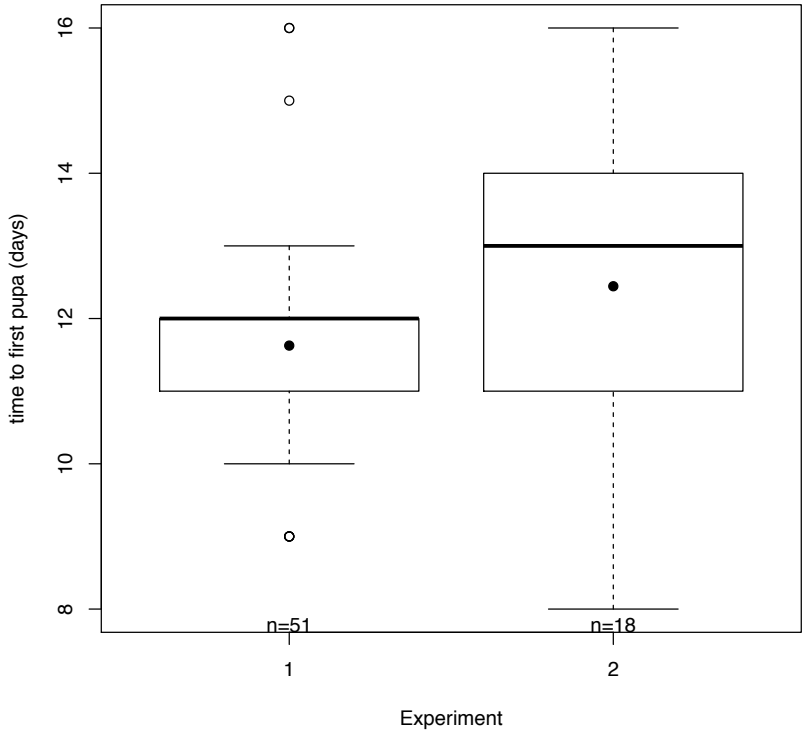

Figure 4.9: Effects of experiment on pupation time.

4.5 Discussion

CONSEQUENCES OF ADULT WORKER SIZE

The results of Experiments 1 and 2 together suggest (1) a weak positive effect of increased adult worker size on new worker size, (2) a weak negative effect of increased adult worker size on the rate of development of new workers, and (3) a weak negative effect of increased adult worker size on brood survival rates.

Overall, the results suggest mild effects of worker size on brood care performance.

These experiments are not conclusive regarding the effect of adult worker size on new worker size but suggest that nanitics may be less efficient in term of production of massive brood. An effect of adult worker size on new worker size was detected in Experiment 1, when nanitics were included in the analysis, but this effect dropped just below significance when nanitics were omitted from the analysis. These results suggest that new colonies that produce nanitics may suffer in their brood care performance relative to colonies of similar numbers of larger workers.

An effect of adult worker size on new worker size may not have detected in Experiment 2 because of the lower food quality in that experiment. *P. badius* castes differ in their N:C composition, with higher N:C ratios in larger (reproductive) castes (Smith & Suarez 2010). Though that study did not detect compositional differences between workers subcastes (minors and majors), size differences among workers may derive from variation in the quality of nutrition experiences during larval stages. In Experiment 2, only seeds were provided, so quality of nutrition was more limited for all colonies, regardless of adult worker size. This idea is supported by the observation that workers produced in Experiment 2 were on average smaller than those produced in Experiment 1. Also, variation in worker size was lower in Experiment 2, and this lack of variation in the dependent variable in Experiment 2 may explain the lack of detection of an effect of adult worker size on new worker size in that experiment. Lack of

variation in a dependent variable can lead to lack of detection of an effect on that variable of an independent variable (Weisberg 1992). Because so many conditions varied between Experiments 1 and 2, additional experiments would be needed to reveal the causes of the conflicting results.

The behavior of another dependent variable, worker developmental time, varied between the two experiments. An effect of adult worker size on new worker developmental rate was detected in Experiment 2, but not in Experiment 1. Experiment 1 may have been limited in its ability to detect determinants of new worker developmental time because there was little variation in time to first worker in that experiment (Figure 4.9). One possible explanation for the lack of variation in time to first pupa in Experiment 1 involves the maturity level of the larvae and the food availability. First, some of the larvae in Experiment 1 were relatively large/old. The critical sizes of Stage C larvae were probably already set. Because food was abundant, these larvae could reach their critical sizes relatively quickly. Critical size achievement triggers the release of prothoracicotrophic hormone (PTTH), which causes pupation. The time to pupation from the achievement of critical size ("PTTH delay time") is relatively constant within a species, plus or minus a day (Nijhout & Williams 1974). Because the larvae used in Experiment 2 were very small, their critical size may have been more plastic during the experiment. This would mean that adult workers (and their size, in particular) could have more effect on the development of new workers in Experiment 2 than in Experiment 1.

CONSEQUENCES OF COLONY SIZE AND LARVAL SIZE

Though this set of experiments was not designed to measure effects of colony size and larval size on the development of new workers, I did detect such effects in Experiment 1, where variance in those random independent factors was considerable. Larval size affected both the development rate and the final size of new workers. Colonies given larger larvae produced the first pupa relatively quickly. This result suggests that, given adequate food and care, the closer a larva is (in age and size) to pupation, the sooner it will pupate. Colonies dealt larger larvae produced smaller workers. If the larger larvae were more mature, they were more likely to be past the stage of setting the critical size. Critical size is a major determinant of an individual's adult size in holometabolous insects, like ants, and it is set early in larval development based on nutritional cues. I would expect the larvae that were initially larger to develop into small workers if they had already set relatively low critical sizes before the experiment began. The critical size of initially smaller larvae, however, may have been malleable to the favorable nutritional conditions of this experiment. Another possible explanation involves the nutrient cycling within social insect colonies. In ant colonies, the 4th instar larvae excrete proteases that help digest animal matter, like the crickets in Experiment 1 (Cassill et al. 2005). As large larvae approach pupation, they would facilitate protein digestion for smaller larvae. These smaller larvae may have had more of their development in front of them and so may have had more of a chance to incorporate the increased protein availability into their final size.

Original colony size also affected both the development rate and the size of new workers. Colonies starting with more adult workers produced larger workers. In two other species of ant, *F. selysi* (Purcell et al. 2012) and *S. invicta* (Tschinkel 1988), larger colonies produced larger workers. This proximate relationship between colony size and brood care is thought to underlie the natural increase in mean worker size with colony size during colony development. Colonies starting with more adult workers also took longer to produce the first new worker. This result is consistent with previous findings in a congener, *P. occidentalis.* New *P. occidentalis* queens with supplemented adult workers produced a first worker a few days later than unsupplemented queens (Chapter 2). In previous studies in other systems, however, brood develop more quickly in larger colonies. In a social wasp, *Polybia occidentalis*, which founds colonies by swarms of workers and a queen, brood developed more quickly in new colonies founded by larger swarms (Howard & Jean 2004). One possible explanation for this discrepancy may have to do with the offspring size. In Experiment 1, larger colonies also produced larger offspring, which may take longer to develop. Porter (1988) found that in *S. invicta*, developmental time is directly related to the size of the developing individual, but there was not a significant relationship between offspring size and offspring developmental time in the present experiment. The extent to which offspring size varies with swarm size during the founding of *Polybia occidentalis* colonies is unknown, but if new *Polybia occidentalis* colonies produce uniformly small workers regardless of colony size, then the extra power of a larger colony might be expected to enhance brood production by

accelerating offspring development in the specific ecological context of that species' colony founding patterns.

CONSEQUENCES OF ADULT:BROOD RATIO

Comparisons between the results of my Experiments 1 and 2 give some insights into the effects of adult:brood ratio on brood care, but these inferences are complicated, given the many other differences in the conditions between the two experiments. In Experiment 1, the adult:brood ratio was 2.7:1, whereas in experiment 2 it was 1:1. My results suggest that a higher adult:brood ratio may allow colonies to produce new workers that are larger and that develop more quickly than those of colonies with a lower adult:brood ratio. However, the colonies in Experiment 1 may have pupated their first larva sooner simply because they began the experiment with some larvae that were larger and so may have been closer to pupation (larvae in Experiment 2 were 2.4 mg on average vs. 3.8 mg in Experiment 1). The adult:brood ratio affects brood development rates in *Formica selysi* (Purcell et al. 2012), with brood developing more quickly under increased adult:brood ratios.

The adult to brood ratio affects offspring size in other systems, and the observed pattern is similar to the one I observed. For example, *S. invicta* brood develop into small adults when the adult:brood ratio is low (Porter and Tschinkel 1985), as in Experiment 2. Brian (summarized in 1957) has also found that the size of new workers increases with an increased adult:brood ratio in *Myrmica rubra*. Purcel et al. (2012) found that colonies of the ant *F. selysi* produced larger

workers when adult:brood ratio was higher, as in Experiment 1. These results are consistent with the present study's result that the workers produced during Experiment 1 (adults:brood ratio 2.7:1) were larger than those produced during Experiment 2 (adults:brood ratio 1:1). Brian (1957) developed a model of the influences of adult:brood ratio and adult worker size on new worker size that incorporates the fact that brood become adults that themselves care for brood. It has been posited that the natural increase in mean worker size with colony size results from the gradual increase in adults:brood ratio (Brian 1957).

I hypothesized that the small size of workers produced early in colony life cost the colony in terms of brood care performance, a short-term, up-close colony growth metric. My results do not support that hypothesis but rather suggest a weak negative relationship between adult worker size and new worker size, at least in the lab, a weak negative relationship between adult worker size and brood development rate, and a weak negative relationship between adult worker size and brood survival probability. That smaller workers produce smaller workers that develop more quickly suggests that brood care in new colonies is not made much less efficient by the fact that the first clutch consists of nanitics vs. non-nanitics. Early colony growth, which may determine colony survival and which depends on brood care performance, appears to suffer little from the small size of nanitics.

Acknowledgements
Thanks to Diane Wiernasz for help in developing the ideas in this chapter and for assistance with data analysis and interpretation. Lulian Ho and David Alvarado helped construct these colonies and maintain them. Lulian and David also helped measure pupae.

5 The consequences of first clutch size and queen size in direct competition between new *Pogonomyrmex barbatus* colonies

Note: This experiment was executed jointly by myself and Lulian Ho.

Diane Wiernasz performed the analyses.

5.1 Abstract

Large offspring are often favored in organisms with high juvenile mortality due to intraspecific competition. This pattern has been documented for organisms as diverse as plants and fishes, but has not yet been described in social insect colonies, which also have high juvenile mortality and indeterminate growth. I asked whether the number of workers in an ant queen's first clutch affected her survival probability under conditions of direct intraspecific competition. I set up matches consisting of pairs of *Pogonomyrmex barbatus* queens. Each queen was given a specified number of workers to compose her first clutch and then put in direct contact with another incipient colony. I found that match outcomes were independent of a queen's first clutch size. In fact, I observed queens fighting each other directly and detected an advantage of relative queen size in match outcomes. In *Pogonomyrmex barbatus*, direct intraspecific competition does not appear to select for large first clutches, or initial offspring colony size. Intraspecific competition may select for large queens, which are analogous to the propagules of plants and some fishes.

5.2 Introduction

In indeterminately growing organisms, such as plants and teleost fish, initial offspring size can affect early survival (fish: Anderson 2002, Sogard 1997; plants: Dalling & Hubbell 2002). In general, the larger the offspring, the better its chance of survival, at least when there are high rates of intraspecific competition. In plants, for example, larger seeds result in initially larger seedlings that have better chances of survival (e.g. Marshall 1986).

Initial offspring size may affect survival in social insects as well, where selection acts at the level of the colony. A colony's fitness is determined by the number of descendant colonies. Colonies begin small and grow in number of workers. Independent colony founding is a common method of colony founding in the social insects (Holldobler & Wilson 1990). In independently founded colonies, or colonies begun by queens without adult workers, initial colony size (number of workers) is by definition very low (it is, in fact, zero). These early, or incipient, colonies, are the equivalent of a teleost fish's larval stage (Figure 5.1). Some social insect colonies share the general life history pattern of fish and plants (they suffer high mortality during early life stages, grow throughout adulthood, live long, and reproduce nearly annually until death) (Gordon 1995; Gordon & Kulig 1996; Cole & Wiernasz 2000). Therefore social insect colonies are good candidates for size-based mortality due to intraspecific competition. The importance of initial offspring size, or first clutch size, to early survival in social insects, however, is unknown.

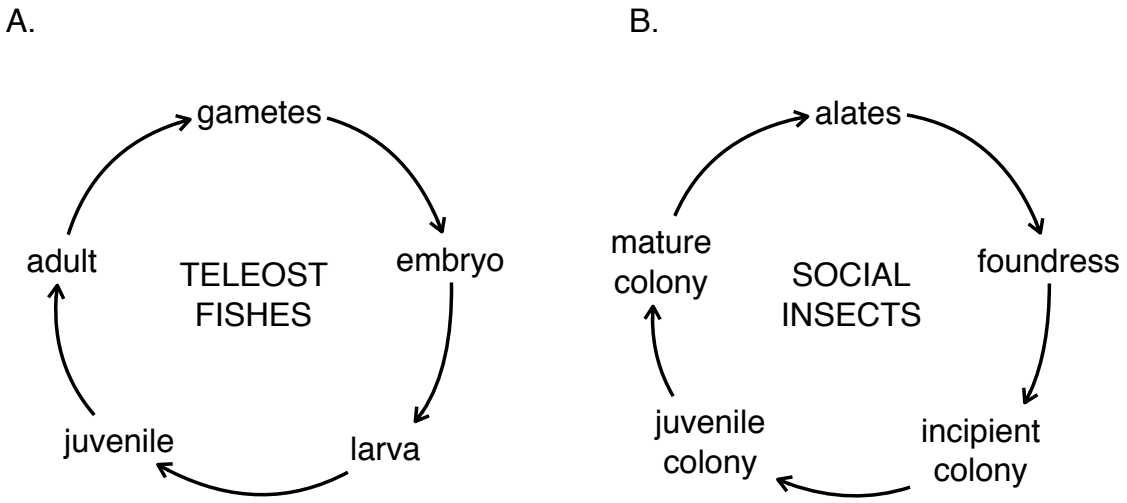

Figure 5.1: Life cycles of teleost fishes and social insects. The life cycle of teleost fishes and of social insects are shown in A and B, arranged so that the parallel stages of life are apparent. For example, the incipient colony stage of social insects is analogous to the larval stage of teleost fishes. In their life histories, social insects and teleost fishes share relatively long lives, high juvenile mortality, and indeterminate growth.

High mortality early in colony life is common in the social insect species that found their colonies independently. Survival has been measured at various stages of early colony life (Table 1). With field surveys of *Solenopsis invicta*, Tschinkel (1992) estimates that 25% of foundresses (or newly-mated queens observed digging a nest) survive to the incipient colony phase (or the eclosion of the first brood of workers), and that 5% of incipient colonies survive to the juvenile colony stage (or through the first months of colony life). In *Pogonomyrmex occidentalis,* 16% of foundresses survived to the incipient colony stage and the colonies of fewer than 1% of foundresses survive to one year (Wiernasz et al. 2003). The survival rate for *Pogonomyrmex barbatus* queens from the time they depart from their parent nest for the mating flight to the young

(1-year-old) colony stage is estimated to be 1% (Gordon & Kulig 1996). *P. barbatus* queens found colonies claustrally; that is, they use internally stored reserves to produce the first clutch of workers.

These high early mortality rates may be caused by various factors. New colonies actually "kill" each other via brood raiding (*S. invicta*), in which the workers from one colony steal brood from another colony. The workers and the queen of the raided colony may eventually join the raiding colony, but usually only one queen ultimately survives (Tschinkel 1992). Queens fight during colony founding (*P. occidentalis*) and some are killed by pathogenic fungi (Wiernasz & Cole, unpublished data). Queens also fight during joint colony founding (pleiometrosis) (*Veromessor pergandei*: Rissing & Pollock 1987). Lizards and birds prey on *P. barbatus* (Gordon & Kulig 1996) and *P. occidentalis* (Wiernasz & Cole 1995). Queens may die from desiccation (Pfenning 1995). Another possible cause of incipient colony death in *P. barbatus* is direct inter-colony competition. *P. barbatus* foundresses often dig nests very close to each other (McCook 1897), so once the first clutch ecloses, it is likely that the two colonies will interact. The interactions between neighboring new colonies may be hostile and even fatal.

In interactions between incipient colonies, I thought that a queen's initial clutch size relative to that of her neighbor might affect her probability of survival. For example, if the workers of the two colonies fight each other to the death one-on-one, the victor identity might depend on first clutch size because the queen that started with more workers in its first clutch would have more workers after the interaction, and so have a greater chance of emerging alive. Another possible

intercolony conflict scenario is that one colony's workers invade the opposing nest and try to kill the queen. The colony with more workers would also be expected to emerge victorious from this kind of conflict, here because it would essentially have more "muscle" with which to battle the queen. If queens battle each other directly, one-on-one, then queen size may be a major determinant of the conflict's outcome. Queen size may also confer an indirect benefit in matches. Queens signal their reproductive potential to workers (Dietemann et al. 2003) and such signals may be stronger from larger queens.

That there may be selection for first clutch size in independently-founding social insect colonies is suggested by the existence of nanitics. Nanitics are the workers composing those first clutches and are universally smaller than workers produced later during colony life. It is thought that when queens allocate their resources during production of the first workers, they favor worker number over worker size. There is support for the value of worker number for brood care performance (Porter & Tschinkel 1986), but initial worker number may have other consequences for new colony fitness.

I designed an experiment to test for an effect of first clutch size on the abilities of new colonies to emerge victorious from competition with each other. Nothing was known about how new *P. barbatus* colonies (each consisting of just the queen and her first clutch) would behave towards each other, so I let pairs of incipient colonies interact and noted their interactions. I varied first clutch size among the colonies and tried to keep queen size constant between paired colonies. I noted the final nest site, queen survival, seed location, and brood

location. Based on my main hypothesis that relative first clutch size affects match outcome, I expected that queens with an advantage in first clutch size would have a greater probability of surviving their matches.

5.3 Materials and Methods

I collected *Pogonomyrmex barbatus* queens immediately following a mating flight in Bear Creek Golf World in Katy, Texas, on June 17, 2012. I brought the queens to the lab, rehydrated them by giving them access to a water-soaked cotton ball for a few hours, and then weighed them on an analytical balance (AT20 Mettler Toledo, Ohio, USA) to the nearest 0.01 mg.

In nature, *P. barbatus* queens found their colonies claustrally; they produce the first batch of workers without foraging. So, after weighing each queen, I placed her in a test tube that had been filled partially with water and plugged with a cotton ball. I stored these tubes in a 30^{o}C incubator in constant darkness until the first workers eclosed. Starting two weeks after the mating flight, I checked the tubes weekly for mature (yellow) pupae.

When adult workers began to appear, on July 18, 2012, I counted each colony's pupae and larvae. In preparation for construction of the experimental first clutches, I removed the pupae from most of the colonies. I used these pupae and the field-collected queens to construct the colonies for this experiment. I shuffled the pupae and then distributed them among queens without regard for their parentage, and so some workers may have ended up in experimental colonies with their own queen mothers.

In this population of *P. barbatus*, natural first clutch size ranges from 0 to 8 (Chapter 3 Figure S1), and there is a trend of initially larger queens making larger first clutches, or first clutches consisting of more workers (original clutch size = 0.05 *queen initial mass + 0.15, R^2 = 0.02, *P* = 0.09, Chapter 3).

I created experimental and "natural" colonies with various first clutch sizes. To make the experimental colonies, I pooled pupae from the first clutches of multiple queens, distributed them among the queens, and started the matches after these pupae eclosed. For the natural colonies, I selected from among all the new queens those which had first clutches of the desired sizes. I let each colony bring its first clutch to maturity before starting the matches.

Some pupae failed to eclose and some larvae matured into adults. As the first clutch eclosed, small adjustments were made to colony composition to get the desired first clutch sizes. I removed workers from experimental and natural colonies and added pupae as necessary to experimental colonies. The composition of some planned matches were changed for feasibility.

I matched the constructed colonies against each other. I made pairwise combinations of one, four, six, eight, and ten nanitics in experimental colonies and pairwise combinations of four, six, and eight nanitics in natural colonies. For example, there were three matches consisting of one nanitic vs. four nanitics. I also set up three matches of two nanitics v. seven non-nanitics. Some of the natural and experimental colonies were paired with colonies with the same number of nanitics (e.g. 4v4, 6v6) (Figure 5.2).

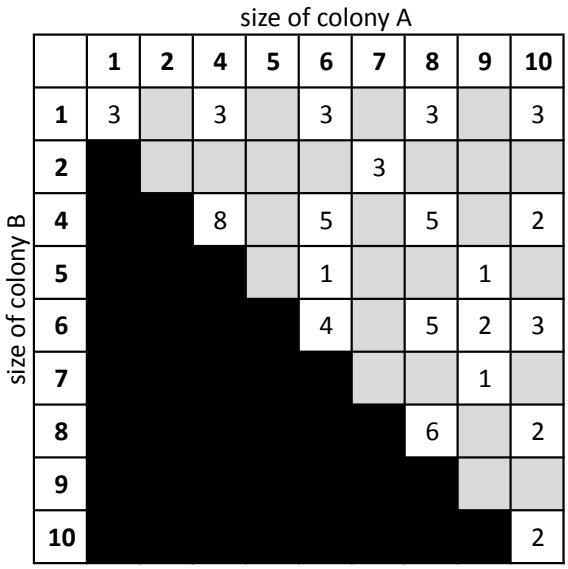

Figure 5.2: Total number of replicate matches for each difference in colony sizes. For example, I set up 3 matches of 4-worker colonies vs. 1-worker colonies.

In general, I tried to match queens by initial wet mass, but queens in some pairs were not evenly matched because I had a limited number of queens. I marked queens and workers with yellow or red Sharpie oil-based paint pens on the back of the head, thorax, and abdomen, alternating colors within size-pairing treatment classes. I applied the paint using a pin tip. Most queens were painted before their first clutch eclosed. Workers were painted up to a day before the start of the matches.

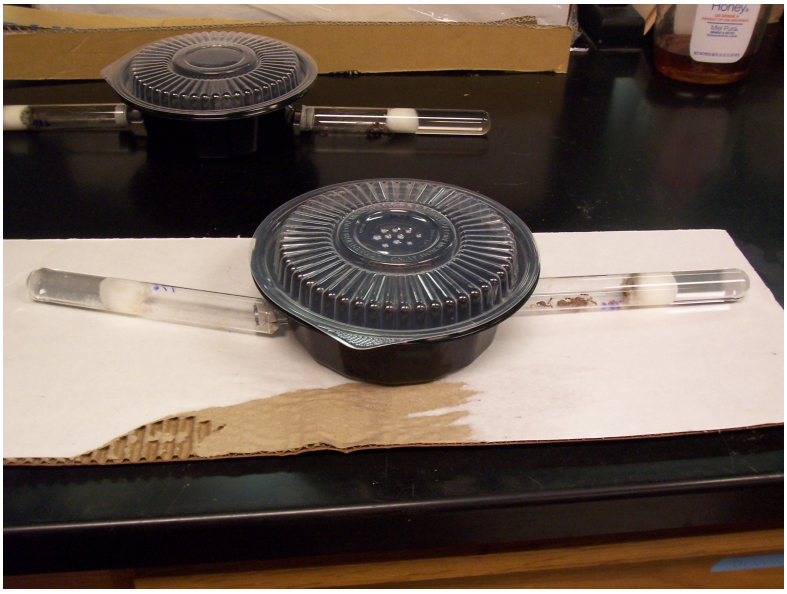

Figure 5.3: Competition arena. Colonies were housed in humidified glass test tubes connected to a common foraging chamber made of a plastic food container. The arenas are shown here on the benchtop, but during the experiment they were kept in incubators on cardboard trays.

Each competition arena was made of a circular plastic food container (11 cm diameter x 4 cm height) with a lid and two test tubes (Figure 5.3). I punched small holes in the top of the food container so air could pass through. The 20 x 150 mm test tubes were the colony nests. The food container served as the foraging chamber. I drilled two holes in opposite sides of the foraging chamber and connected the tubes to the foraging chamber through these holes using rubber tubing covered in foam window tape for a snug fit. I kept the complete arenas on cardboard trays on wire shelves in an incubator in constant darkness at 30^{o}C. I put two pieces of cracked wheat and two pieces of sunflower seed in the foraging chamber of the arena before the colonies were connected to it.

The experiment was run as three sets of matches, started on 8 August, 12 August, and 27 August. This is because not all clutches eclosed simultaneously, and I wanted to start as many matches as soon after the mating flight as possible, so that the queen's biochemistry during the match is close to what it would be in nature in incipient colonies.

I noted the locations of seeds, brood, workers, and queens one and two days after the start of each match (nest 1, nest 2, or competition chamber). I also noted the status of the queen (intact, missing legs, dead). After the second day of a match, I checked the match daily for resolution in queen death. Not all colonies fought to queen death, and I defined indecisive matches as those in which, at the end of 10 days, both queens were still alive and intact. In some colonies, both queens died. These were scored as double loss.

Although my hypothesis was framed in terms of clutch size, and I made an effort to equalize the size of competing queens, it is possible that queen size differences could also contribute to the outcome of this experiment. I analyzed the data using logistic regression (Systat 11, Wilkinson 2004), using as the focal colonies those with yellow queens. I tested whether the difference in colony size (number of workers) and the difference in queen mass determined whether the yellow queen would "win" (i.e., survive). I excluded all trials that ended as a double loss (both queens died) or were inconclusive (both queens alive at the end of ten days). My hypothesis was that larger size of either the colony or the queen would provide an advantage, so I used one-tailed tests.

5.4 Results

Table 5.1: Summary of match outcomes

	Natural colonies	Experimental colonies
indecisive	2	3
decisive	19	40
double loss	3	0
total	24	43

Most matches ended with the death of one of the two queens (Table 5.1). At the end of most decisive matches, all live ants were in one nest (Figure 5.4). The workers whose queens survived were not necessarily in their native nests at the end of their matches. In most cases, the brood and seeds were all in one nest a day or two after the start of a match. A few days later, most of the adults in a match were together in one nest, and one queen was abandoned either in her nest or in the arena. Usually damage to that queen occurred later.

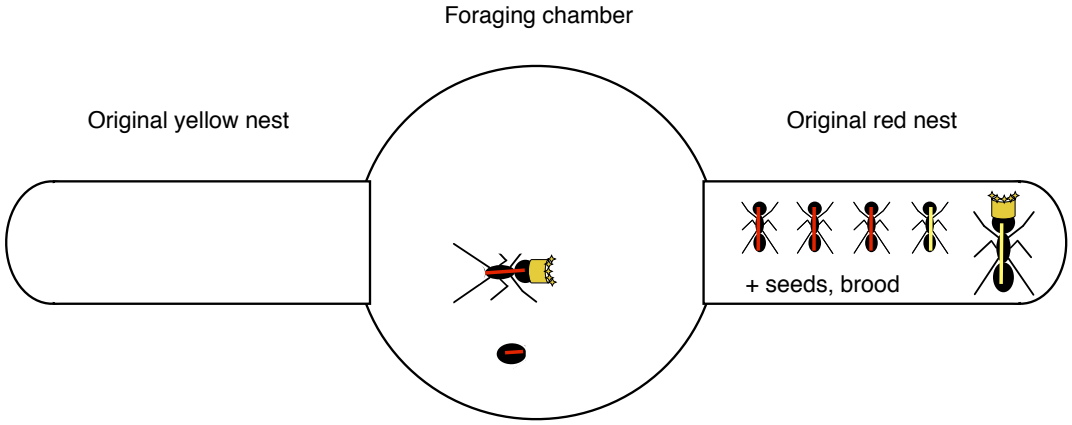

Figure 5.4: Example of a common outcome. Here all ants alive at the end of the experiment were found in the original red nest even though the red queen was dead. There was no clear relationship between final nest identity and identity of the victor queen across matches.

Workers attached themselves to queen legs, presumably by biting them. Some queens fought each other directly, clamping onto the petiole of the other queen. Some workers clamped on to each other's petioles in fights and dragged each other around, sometimes between the nests and the foraging chamber. The queens survived a surprisingly long time even after sustaining the loss of multiple limbs. Usually, though, if only one queen in a match was missing limbs, that queen was more likely to be the one to die during the 10 days of the experiment.

Larger queens were significantly more likely to survive than smaller queens, regardless of the size of their colony (t ratio = 2.18, $P = 0.0146$) (Figure 5.5), but the relative size advantage of the colony did not significantly affect the outcome (t ratio = -0.891, $P = 0.187$) (Figure 5.6). Large queens were approximately 20% more likely (per increment of mass difference) to win a trial (log of odds = 1.278, C.I. = 1.031 – 1.589).

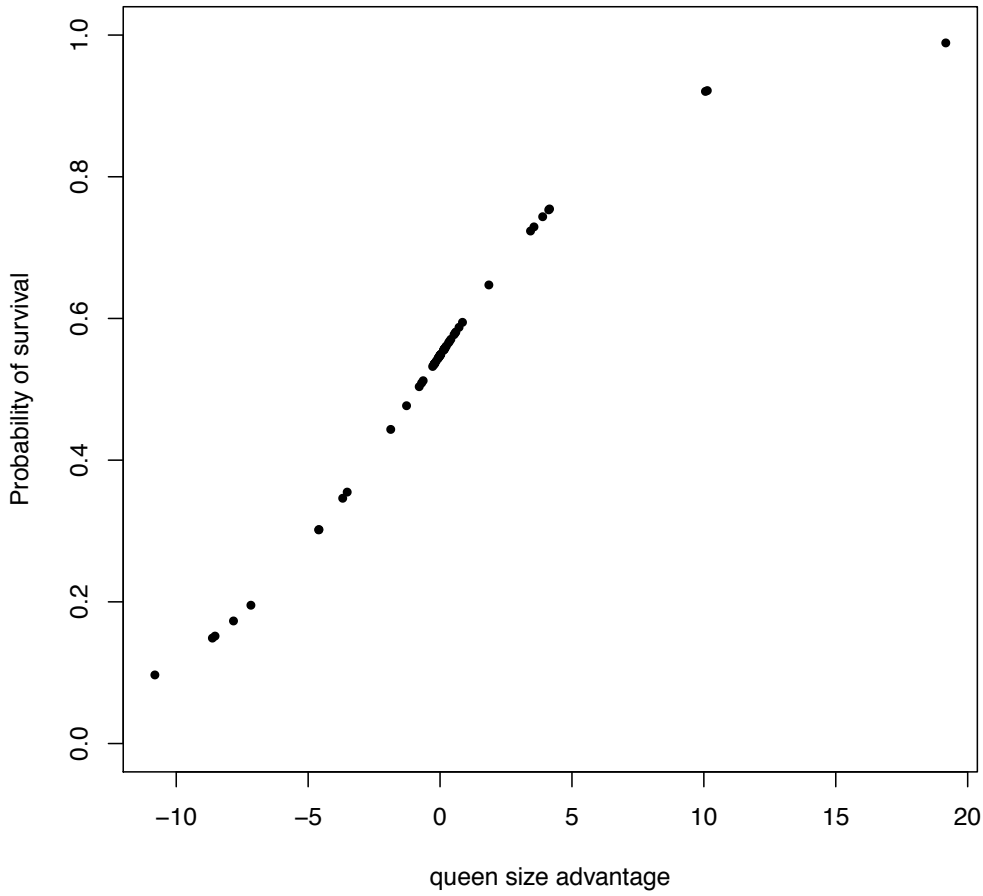

Figure 5.5: Queen size and the probability of queen survival. The x-values of the displayed points are the actual queen size advantages. The y-values were calculated using the logistic regression model that was fit to the actual survival data.

size of colony A

size of colony B	1	2	4	5	6	7	8	9	10
1			0		0		2		2-
2						2*-			
4	3-				4-		1-		1
5					0			1	
6	2		0	1-			2	0	1
7		1*						1*	
8	1		3*		2				1
9				0	2*	0			
10	0		1*		2*		1		

↑ wins by larger colony

wins by smaller colony ←

KEY

* smaller colony had much larger q (>3 mg diff) in at least one case

\- larger colony had much larger q in at least one case

Notes

1 tie 4v6
1 tie 8v6
1 tie 4v8
1 tie 6v1
1 tie 10 v 1

These data were INCLUDED above

	2	7
2		2
7	1	

	4	6	8
4		2	1
6	0		1
8	1	0	

Figure 5.6: Match outcomes according to first clutch size. The sizes of paired colonies (A and B) appear along the top (A) and left (B) edges of the grid. Cells represent matches between colonies of the intersecting sizes. Above the diagonal, the number in the cell is the number of times the larger of the two colonies in a match won the match. Below the diagonal, the number in the cell is the number of times the smaller of the two colonies won the match.

5.5 Discussion

The low number of replicate matches per colony size pairing made it hard to read from the results anything definitive about the effects of colony size advantage on the outcomes of competitions. However, colony size does not appear to determine match outcomes. The range of colony sizes tested was beyond the natural range of zero to eight (Chapter 3), so these results suggest that selection for greater first clutch size via intracolony competition in nature is unlikely.

The observed advantage for queens who are larger than their competitors in direct competition in the present experiment suggests an additional selective advantage of queen largeness beyond that previously discovered for the pre-incipient colony stage. Larger queens may have succeeded in this experiment by attracting the workers of the opposite colony, essentially recruiting them away from their native queen. Their size may also have helped them in the one-on-one queen battles. Since queens were observed directly fighting each other, I think that queen death was usually caused by direct queen-queen fights,

It was surprising that some colonies coexisted in separate nests and some colonies even merged and lived peacefully together for the duration of the experiment (five indecisive matches in Table 5.1). The colonies were killed soon after the end of the experiment and both queens in the indecisive matches survived to that point. Preliminary tests with older workers and their own queens resolved in peaceful merged colonies too. I gave one of the test colonies to a colleague and he says that in that colony, the queens sat near their own egg piles at opposite ends of the nest for about two weeks, at which point one queen

began to fall in line with the workers. The “loser” queen in that case was missing some legs. Temporary polygyny in *P. barbatus* is therefore possible, though whether or not queens would normally find themselves in the situations like those that led to the polygyny observed in this experiment is not known. Colony founding by groups of queens has been found in certain populations of a congener, *P. californicus* and is thought to be favored in those sites by high levels of interspecific competition (Johnson 2004). Queens also group together to found colonies in some populations of another harvester ant, *Messor pergandei* (Rissing and Pollock 1987). Cooperative founding can increase a queen’s chances of survival of the initial stages of colony founding. However, in some populations of some cooperatively founding species, only one queens per nest survives the juvenile colony stage (Choe and Perlman 1997).

This work is similar to experiments explicitly investigating the brood-raiding behavior of new *Solenopsis invicta* and *Myrmecocystus mimicus.* Young colonies of these species can steal each other’s brood (Bartz and Holldobler 1982; Tschinkel 1992). The first clutches of *S. invicta* are 8-30 workers compared to the 1-6 of natural *P. barbatus* and the minimum colony size difference in experimental matches in the fire ant study was greater (10 workers) than that in the present study. Tschinkel defined match victory as all brood being in one nest. He found that colonies with larger first clutches won more matches. Bartz and Holldobler constructed matches with smaller first clutches and smaller differences in first clutch size and found that in most cases the colonies with the greatest initial clutch size gathered all of the brood of the other colonies into their

nest. These results support the hypothesis that at least in species with brood-raiding, production of a relatively large first clutch of relatively small workers may be adaptive. However, since neither of these studies followed all colonies through queen death, it is not clear whether the brood-raiding victory constitutes a victory in terms of queen survival and therefore fitness. Tschinkel found that "loser" queens often joined the winning nest, but the chance of these queens to survive the colony's subsequent queen reduction (to a single reproductive queen) is unknown.

Acknowledgements
I thank Diane C. Wiernasz for analyzing the data. This work was made possible by a grant from NHARP to Diane Wiernasz. Queen collection was successful because of Jessica Tarrand and Yen Saw. David Alvarado made the foraging arenas and Stephanie Rice helped paint the ants.

6 Ecology and evolution of the early development of social insect colonies

Mortality peaks early in the lives of many species (benthic marine invertebrates: Gosselin & Qian 1997; spiders: Moreira & Del-Claro 2011; snails: Keller & Ribi 1993), so it is important to examine selection's impact on early developmental patterns. Selection often acts indirectly on juvenile form, directly favoring increased growth rate (out of the juvenile stage). In this dissertation, I asked, "Why do individuals progress through the beginning stages of life in the manner that they do?" I broke this question into two subquestions (1) What aspects of development are proximately linked and what limiting "resource" links them? (2) What are the fitness consequences of a given growth pattern? Social insect colonies were an excellent subject for the study of developmental phenotypes because the developmental units of a colony (the workers) are biological individuals. Though it is clear that colonies of some species benefit from the standing worker size variation present during colony adulthood (e.g. Billick et al. 2007, Haight 2010), little was known about the fitness consequences of the small size of the first workers produced by all colonies which are founded independently, by queens without adult workers.

I first approached this dissertation's central question "Why do new colonies make small workers?" with a reductionist mindset. In social insects, adult body size is set in part by the rate of provisioning in the larval stage (Wheeler 1994). I therefore hypothesized that new colonies make small workers

because the circumstances of colony founding limit the rate at which their larvae are provisioned. The experiment described in Chapter 2 was designed to identify the aspects of new colonies that limit the rate of larval provisioning. New worker size (and other aspects of worker production) was most affected by the presence of adult workers. Though I did not directly measure larval provisioning rate, this result is consistent with a hypothesis that new colonies produce small workers because there is only one caretaker, the queen.

A proximate explanation, like the one established in Chapter 2, simply describes the response of a trait to an environmental factor; it does not address the origin of that response curve. The other main way to answer questions in Biology fills this gap: ultimate explanations describe how trait values affect fitness. Ultimate explanations use the consequences of traits for fitness to paint a picture of how, through generations, the trait of interest may have come to be. The experiments of chapters 3-5 were designed to measure the consequences of a growth pattern; the size and number of workers that the queen produces to start her colony. Because my trait of interest, worker size is linked physiologically to clutch size, I analyzed their consequences together. Colony growth rate was used as a fitness metric because field studies have linked colony growth rate to new colony survival. Initial clutch size, but not worker size, emerged as a determining factor in the eight weeks of colony growth measured in Chapter 3; initially larger colonies grew faster. The results of these two experiments are consistent with the hypothesis that selection favors investment in clutch size over worker size during colony founding.

The experiments of Chapter 4 were inconclusive; they neither rule out nor support the possibility that worker size can affect the production of a single batch of workers. Some issues with the experiments were uncontrolled variation in key parameters (e.g. initial larval size) and low sample size (in Experiment versions 1 and 2, respectively). Still, the results of these experiments suggest that if there is a cost of the small size of nanitics to brood care performance, it is probably small. With a bounty of newly-mated queens and some older stock colonies from which to harvest non-nanitic pupae, one could more directly test for a cost of the small size of nanitics on brood care on a short time scale during colony founding, on production of the second clutch.

Chapter 5 described an exploration of another possible consequences of first clutch size for fitness, specifically, the consequences of initial worker number (first clutch size) for survival of competition between new colonies. First clutch size did not affect the probability of victory in competition, but queen size did. These results argue against the importance of first clutch size for survival of intraspecific competition in *P. barbatus*.

The increased first clutch size with the presence of adult workers (in Chapter 2) and the increased growth rate of colonies with increased first clutches (in Chapter 3) both emphasize the self-catalytic role of workers in social insect colonies. Though the queen actually lays the eggs that become workers, it seems that in many ways, workers beget workers. In other words, growth of the colony's worker population, or soma, depends on the colony's size. Size-dependent growth rates also occur in plants, where larger hatchlings are more

competitive in the fight for resources (e.g. Stanton 1984). Similarly, resource availability can be affected by colony size, where having more workers can lead to having more food (Cole et al. 2008).

My results are consistent with the idea that the production of nanitics by new independently-founded social insect colonies is part of an adaptation to high mortality during colony founding. As for some seedlings and fish larvae, new colonies may be best analyzed ultimately not according to their static form (worker size or even first clutch size *per se*), but rather for a dynamic trait, growth rate. Previous work has demonstrated that initial size can matter for growth rate and that growth rate can matter for survival in multiple branches of the phylogenetic tree (marine invertebrates: Marshall et al. 2003, plants: Stanton 1984, teleost fishes: Sogard 1997). The work described in this dissertation suggests that with only a slight change in the definition of an individual (from a unitary individual to a colony) this kind of natural selection may explain traits in social insect colonies as well.

This work could be fruitfully expanded through an exploration of (1) the scaling of selection for colony growth rate with colony size and (2) the scaling of the effect of clutch size and worker size on colony growth rate. In other words, we could ask “How does the magnitude of selection for colony growth rate change over a colony’s lifetime, as the colony increases in size?” and “How do the affect of clutch size and worker size on colony growth rate scale with colony size?” If production of nanitics is an adaptation to high juvenile mortality, then we should see selection for growth rate and/or the effect of clutch size on growth

rate decline with colony size. If, instead, selection for high growth rate continues throughout colony development (with increases in colony size), then it makes more sense to ask why large workers are made later in colony development than why small workers are made early. One possibility is that because larger workers have greater intrinsic lifespans than small workers (Calabi and Porter 1989), colonies that make larger workers may grow (i.e. increase in the number of workers that they contain) faster over relatively long time scales (years vs. the weeks that matter during colony founding). We would need to know the actualized longevity differences among workers of different sizes and the seasonal schedule of worker production to confirm or discredit this hypothesis.

7 References

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