

LIFE HISTORIES AND ENERGETICS OF BUMBLE BEE (*BOMBUS IMPATIENS*)
COLONIES AND WORKERS

by

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I dedicate this to my wonderful husband and soul mate, Tuan Cao. His unconditional love, patience, and support mean the entire world to me.

TABLE OF CONTENTS

ABSTRACT.....	8
INTRODUCTION.....	10
PRESENT STUDY.....	20
REFERENCES.....	23
APPENDIX A.	
PRIORITY RULES OF ENERGY ALLOCATION AT THE GROUP LEVEL: HOW BUMBLE BEE COLONIES ALLOCATE ENERGY WHEN RESOURCE AVAILABILITY VARIES.....	27
APPENDIX B.	
EFFECTS OF RESOURCE AVAILABILITY ON WORKER SIZE DISTRIBUTION IN BUMBLE BEE (<i>BOMBUS IMPATIENS</i>) COLONIES.....	59
APPENDIX C.	
WHAT IS A BEE'S WORTH? THE PRODUCTION COSTS AND WORK OUTPUT OF DIFFERENT SIZED BUMBLE BEE (<i>BOMBUS IMPATIENS</i>) WORKERS.....	85
APPENDIX D.	
METABOLIC RATE DECREASES WITH AGE AND AFFECTS THE LIFESPAN OF BUMBLE BEES, <i>BOMBUS IMPATIENS</i>	115

ABSTRACT

Social insect colonies are complex systems with emergent properties that arise from the cooperation and interaction amongst individuals within colonies. By dividing reproduction and physical labor amongst them, individuals contribute to the growth and ecological success of their colonies, a success that is greater than individuals could achieve on their own. A key characteristic of social insects is a division of labor amongst workers that is determined primarily either by age, morphology, or dominance. Social insects are considered one of the most ecologically successful groups of organisms on earth. Colony life cycles include: 1) growth, in which workers are produced, 2) reproduction, in which queens and males with reproductive capabilities are produced, and 3) senescence.

In life history theory, phenotypic plasticity (i.e. a change in phenotype in response to an environmental change), allows organisms to adjust and optimize fitness in response the change in environments. Central to life history theory is the idea that traits have costs and benefits. Using an energetics framework that considers the costs and benefits of traits contributes to our understanding as to why organisms exhibit the sets of traits that they have within their ecological environments.

Using the annual bumble bee *Bombus impatiens*, my dissertation investigates the effects of resource availability on worker production and on the relative allocation of energy towards growth and reproduction within colonies. Bumble bees have a morphological division of labor and concomitantly, they show large intra-colony size variation amongst workers. Because body size is an important life history trait, I also examined the costs and benefits of producing various sized workers. Lastly, I examined

the association among worker body size, metabolic rate (a measure of maintenance costs), and lifespan.

INTRODUCTION

An explanation of the problem and review of literature

Sociality

Sociality presents a major evolutionary transition from single organisms to colonial units (Maynard Smith & Szathmary 1995; Szathmary & Maynard Smith 1995). The continuum for sociality ranges from primitively social to highly social, or ‘eusocial’. Colonies that possess the following three characteristics are considered eusocial: 1) a division of reproduction, with few individuals who have reproductive capabilities (female queens and males) and many non-reproducing individuals called workers, 2) two or more generations of adults living in the same nest, and 3) the workers participate in brood care (Holldobler & Wilson 2009b). Some examples include snapping shrimp, naked mole rats, termites, bees, wasps, and ants. Eusociality evolved independently 12 times in the arthropods throughout evolutionary history (Wilson & Holldobler 2005), and occurs in 15 of the approximately 2,600 recognized arthropod families (Wilson 1971; Michener 1974; Gadagkar 2001; Wilson & Holldobler 2005). Eusocial insect colonies are often referred to as ‘superorganisms’ which are societies with physiological features analogous to those of individual organisms (Holldobler & Wilson 2009b). Similar to how multi-cellular organisms are made up of differentiated cells, each social insect colony is made up of differentiated individuals. Queens and males are the reproductive individuals, analogous to gametic cells, while non-reproducing females make up the worker caste in Hymenoptera and are analogous to somatic cells.

Whether you look up towards the canopies or down towards the ground, you will surely see many social insects, most notably termites, wasp, ants, and bees. Despite the

fact that they make up only about 2% of the approximately 900,000 recognized insect species, about 50% of the total insect biomass is comprised of social insects (Wilson & Hölldobler 2005; Hölldobler & Wilson 2009b). Of those social insects, most belong to the order Hymenoptera (wasps, ants, bees) while a small portion belong to the order Isoptera (termites) (Wilson 1990). Social insects represent a level of biological organization that is above the whole organism and below that of the ecosystem. This, in consideration with their large presence on earth, makes social insects useful and accessible for understanding how individual units interact to create emergent patterns (Hölldobler & Wilson 2009b).

How exactly do social insect colonies achieve such ecological success? Part of their ecological success is due to the many benefits of colonial living, such as shared nests and food sources, cooperative nest defense (Hölldobler & Wilson 2009b; Nowak, Tarnita & Wilson 2010), and controlled interior microclimate within the nest (Hölldobler & Wilson 2009b). Their amazing success is also due to their ability to grow and reproduce as cohesive colonial units that are made of tens, thousands, or even millions of related individuals (Hölldobler & Wilson 2009b). Rather than each individual partaking in reproduction and rearing brood, individuals within the nest cooperate by dividing reproduction and labor, allowing the colony to successfully grow in large numbers and produce more (related) offspring than an individual could rear on his/her own. Social insect colonies also have division of labor amongst the workers. For instance, some workers forage while others tend to larvae or defend the nest. Depending on the species, division of labor is determined primarily by age, morphology (Beshers & Fewell 201), or dominance (West-Eberhard 1969). Colony organization and the emergent properties that

we see (e.g. thermoregulation, reproduction, nest defense, etc.), arise from social interactions amongst individuals within the nest (Hölldobler & Wilson 2009b).

Furthermore, just like whole organisms, it is also the way in which colonies respond to their changing environments that promote evolutionary success. Being able to successfully budget energy resources to various colonial needs is an important trait, especially when resources are limited.

Life history framework

Life history theory extends from the branch of evolutionary ecology (Stearns 2000). It is a framework used to understand how species' life history traits evolve within their environments so that they achieve optimal fitness (survival and reproduction). Life history traits are those that define an organism's life cycle (Nylin & Gotthard 1998a), and examples include lifespan, growth rates, body size, and fecundity (Nylin & Gotthard 1998a; Zera & Harshman 2001c). One can think of an organism's life history as an adaptive set of anatomical, behavioral, and physiological strategies (Ricklefs & Wikelski 2002). Ideally, an organism should maximize each aspect of its life cycle; it should have the fastest growth rate, longest lifespan, largest body size, and highest fecundity, eventually leading to a 'darwinian demon' (Reznick, Nunney & Tessier 2000b). This, however, is not the case because of the variation that is observed in populations. This variation arises from trade-offs between or among the life history traits (Reznick 1985; Roff 1992a; Stearns 1992; Zera & Harshman 2001c) and phenotypic plasticity (Nylin & Gotthard 1998a).

Life history traits trade-off when they are phenotypically or genetically negatively correlated with each other (Stearns 1989; Zera & Harshman 2001c). Trade-offs arise

from genetic, phylogenetic, physical, or ecological constraints (Roff 1992a; Stearns 2000; Zera & Harshman 2001c) such as predation (Reznick, Bryga & Endler 1990), behavior that results in increased energy expenditure (Marler *et al.* 1995), or nutrient acquisition (Boggs & Ross 1993; Zera & Harshman 2001c). There are about 45 described trade-offs between life history traits, for example between: survival and current reproduction, offspring quality and quantity, and reproduction and growth (Stearns 1992). A trade-off is often associated with the idea of ‘costs’ to having an increase in a life history trait. These costs are measured in various ways: as a reduction in fecundity as a result of increased growth or a reduction in lifespan due to increased fecundity (Reznick 1985). Costs can also be measured in energy terms (joules or calories). A major ecological theme in life history theory is that of nutrient acquisition and how limited internal resources could lead to differential allocation of energy resources to competing traits within organisms (Chippindale *et al.* 1993; Nijhout & Emlen 1998; Zera & Harshman 2001c). Energy budgets are used to quantify how, with a given amount of energy input, energy is allocated amongst various functions within the organism (Davies & Hatcher 1998). In addition, energy budgets allow us to compare the relative investments towards life history traits using the same units.

When traits respond differently under various ecological conditions, plasticity in trade-offs can occur in which trade-offs are stronger under certain conditions and even obviated under another (Tatar & Carey 1995; Warkentin 1995; Zera & Harshman 2001c; Simmons & Emlen 2006). For example, nutrition influences the trade-offs between mortality and reproduction in the beetle *Callosobruchus maculatus* such that beetles with food available during high egg production showed about a 12% decrease in the trade-off

between mortality and reproduction compared to those without food during high egg production (Tatar & Carey 1995). Priority rules determine the relative allocation of energy resources among traits within organisms as a function of the amount of energy input (Zera & Harshman 2001c). For instance, when resources are limited for the freshwater clam *Anodonta piscinalis*, relatively more energy should be allocated towards maintenance, followed by reproduction as of second importance, and then somatic growth (Jokela & Mutikainen 1995b). Plasticity in life history traits allows organisms to optimize their fitness by adjusting relative energy allocations to multiple competing traits.

Growth, reproduction, and body size

The trade-off between growth and reproduction is well-studied. With reference to energy resources, organisms fall within a continuum of breeding strategies that ranges between capital and income breeding. Capital breeders use stored energy reserves to produce offspring while income breeders obtain energy resources concurrently with reproduction (Johnson 2006; Stephens *et al.* 2013). Organisms are also either semelparous or iteroparous. Semelparity, or ‘big bang’ reproduction, refers to the pattern in which organisms breed once and then die (e.g. Pacific salmon and mayflies) while iteroparous organisms breed more than once within their lifetimes (Bell 1980; Roff 1992a). Regardless of the type of breeder or reproduction strategy, reproduction is energetically costly (Reznick 1985). When organisms reproduce, a decision is made on how to allocate energy resources towards reproduction itself versus other traits (Heino & Kaitala 1999). Environmental stress, such as competition or resource limitation, can affect the relationship between growth and reproduction (Jokela & Mutikainen 1995b; Petes, Menge & Murphy 2007). Reproduction is associated with growth through

interactions among development time, growth rate, and adult size (Roff 2000). Typically, a positive correlation exists between body size and fecundity (Blueweiss *et al.* 1978; Roff 1981; Roff 1992a; Honěk 1993; Lyimo & Takken 1993; Roff 2000), and body size is generally, but not always, positively correlated with growth rate and development time (Angilletta, Steury & Sears 2004).

Body size is inarguably an important life history trait (Calder 1996; Bonner 2006). In addition to being positively correlated with fecundity (Blueweiss *et al.* 1978; Roff 1981; Roff 1992a; Honěk 1993; Lyimo & Takken 1993; Roff 2000), larger individuals also collect more food (Alcock 1979; Johnson 1990; Goulson *et al.* 2002), forage at higher rates (Spaethe & Weidenmüller 2002), and have longer lifespans (Speakman 2005b). However, body size variation exists in populations. Although there is overwhelming evidence of the fitness benefits associated with being larger, large body size comes at a cost. These costs include high maintenance costs (i.e. overall metabolic rates), reduced agility, and increased visibility (Blanckenhorn 2000). Thus, selection could also favor small body size. For instance, small size in Dipteran males is related to higher success in mating, probably due to better agility (McLachlan & Allen 1987; Steele & Partridge 1988). Small organisms also cost less energy resources to produce, as less food provisioning and energy would be involved in rearing small organisms, and this would be advantageous to provisioning parents (Bosch & Vicens 2002). Body size variation in populations therefore results from trade-offs with other life history traits and is a reflection that organisms utilize different life history strategies in order to optimize fitness within their environments.

Social insects within a life history context

Social insect colonies go through life cycles that include growth, reproduction, and senescence (Goulson 2003a; Hölldobler & Wilson 2009b). Some social insect colonies are perennial and may have large food stores (e.g. ants or honeybees) while others are annual (e.g. some bumble bees) and have less food stores. Because resource limitation is a common problem for colonies in nature, a part of my dissertation investigates the effects of resource availability on growth and reproduction. More specifically, my dissertation examines growth and reproduction in an annual species, in which colonies have one chance to attain reproductive success within the flowering season. Considering that annual colonies do not have long-term food stores, resource limitation may have strong effects on colony growth and reproduction. There are studies on how resources affect reproduction and growth (Herbers & Banschbach 1999a; Aron, Keller & Passera 2001a; Foitzik, Strätz & Heinze 2003; Smith 2007a; Elliot 2009), but these studies were conducted either on perennial ant colonies (Herbers & Banschbach 1999a; Aron, Keller & Passera 2001a; Foitzik, Strätz & Heinze 2003; Smith 2007a) or did not compare the relative growth and reproduction within colonies (Elliot 2009). To my knowledge, how resources affect the *relative* allocations to growth and reproduction *within* annual colonies is not known. The relative comparison between growth and reproduction within colonies tells us whether annual colonies can strategize their energy allocation in response to changes in resource availability.

Because body size is an important life history trait, my dissertation also examines how body size influences colony-level worker production. An individual's body size in a social insect colony is different from non-social organisms because the individual is part of a superorganism. Body size is especially important for social insect colonies with a

morphological division of labor because task performance is associated with body size (Goulson *et al.* 2002; Hölldobler & Wilson 2009b). Concomitantly with a morphological division of labor, there is large intra-colony size variation among workers (Alford 1975; Wilson 1980; Hölldobler & Wilson 1990; Araujo & Tschinkel 2010). As I discussed earlier, size variation reflects costs and benefits associated with being of particular size. Although the benefits of a morphological division of labor have been studied in terms of behavioral efficiencies associated with particular sizes (Wilson 1980; Goulson *et al.* 2002; Spaethe & Weidenmüller 2002), there has not been a report on the energetic costs and benefits related to worker size. I believe that an energetic cost and benefit approach could add to our understanding of why large variations in worker size exist within social insect colonies that have a morphological division.

Within a life history context and an energetic cost and benefit approach, my dissertation examines whether colonies can adjust aspects of their life cycles to their environment. I also examine individual-level costs and benefits of workers and discuss how these individual-level traits influence colony-level growth and division of labor.

Study organism

I used the bumble bee, *B. impatiens*, as my study organism. *B. impatiens* colonies are annual and can live for several months under laboratory conditions (personal observation), allowing me to keep complete track of colony growth and reproduction, from initiation to senescence. I could also easily collect workers to measure their body mass and the energy content of their body size via bomb calorimetry to assess worker production costs. I could also easily follow workers throughout their lives and record their lifetime work efforts. *B. impatiens* queens are usually singly mated (Schmid-

Hempel & Schmid-Hempel 2000a) and like all Hymenoptera, bumble bees are haplodiploid. Thus, workers within each colony are highly related (average coefficient of relatedness (r) = .75) (Hölldobler & Wilson 2009b). A single colony begins with one individual and expands in population size throughout the growing season. Colony founding begins when a mated queen emerges from overwintering diapause and rears the first set of workers by herself. After the first set of workers emerges, they help the queen rear the rest of the colony's population. All colonies go through a growth phase in which only female workers are produced. The growth phase is followed by a reproductive phase in which worker production slows down significantly and eventually halts while virgin queens (larger females) and males are produced. Colonies are thus semelparous. Queens and males would then leave the nest to mate; their natal colonies would senesce and eventually die. Mated queens would then overwinter and found their own colonies the following spring (Heinrich 1979; Goulson 2003a). I consider colonies to be an intermediate between capital and income breeders, since they can store some food but only for short periods of time. In bumble bees, division of labor is associated with body size, where larger bumble bee workers are usually foragers and smaller workers perform within-nest tasks (Goulson *et al.* 2002; Jandt & Dornhaus 2009a), although workers can switch among tasks (Jandt, Huang & Dornhaus 2009b).

Explanation of dissertation format

In this dissertation, I investigated individual and colony-level life history traits in the bumble bee, *Bombus impatiens*. Specifically, I investigated: 1) the effects of resource availability on relative allocations between growth and reproduction, 2) the effects of resource availability on worker size distribution, 3) the association between energetic

costs of worker size and lifetime work output, a measure of behavioral benefits for colonies, and 4) the association among body size, metabolism, and adult lifespan in workers. Overall, my dissertation examines whether colonies can adjust growth and reproduction to their resource conditions. I also examine individual-level costs and benefits of workers and discuss how these individual-level traits influence colony-level growth and division of labor.

This dissertation is presented in four appendices, each formatted as a manuscript. Appendix A examines the effects of resource availability on the relative allocation between growth and reproduction within colonies. Appendix B examines the effects of resource availability on worker size distribution within colonies. Appendix C examines the association among body size, production costs, and lifetime work output. Appendix D examines the association among body size, mass-specific metabolic rate, and lifespan.

PRESENT STUDY

The methods, results, and conclusions of this study are reported in the manuscripts appended to this dissertation. The following is a summary of the most important findings in this document.

Appendix A examines the effects of resource availability on the relative allocation between growth and reproduction within colonies. I manipulated resource availability for six *B. impatiens* colonies such that colonies received either limited or unlimited resources. Since body mass should be positively correlated with production costs, I measured energy allocation towards colony growth as the total biomass of workers that colonies produced. Similarly, I measured energy allocation towards reproduction as the total biomass of all the queens and males that colonies produced. I found that when resources are limited, colonies invested relatively more energy resources into growth than into reproduction. When resources were unlimited, colonies invested relatively more energy resources into reproduction. I also found that growth rates in colonies were marginally faster when resources were unlimited, allowing colonies to initiate reproduction earlier. My results show that it is possible for colonies to adjust relative energy allocations towards growth and reproduction, presumably so that colonies can optimize fitness under different resource conditions. Overall, my results show that bumble bee colonies have priority rules for energy allocation such that when resources are limited, growth takes priority over reproduction.

Appendix B examines the effects of resource availability on worker size distribution within colonies. Similarly as in Appendix A, I manipulated resource availability for six colonies such that colonies received either limited or unlimited

resources. I measured the skew, kurtosis, and variation of body mass for newly emerged workers throughout the beginning, middle, and end of the colony growth period (period in which colonies produce only workers). I also measured the skew, kurtosis, and variation of body mass for all of the workers that colonies produced. I found that resource availability did not affect the distribution shape (skew or kurtosis) or size variation of mass for all workers that colonies produced. This was also true for the body mass distributions for newly emerged workers produced throughout colony growth. In addition, I found that the size distributions of newly emerged workers produced throughout colony growth were usually, but not always, normally distributed. Those that were not normally distributed were skewed to the right. Overall, my results show that the shape and variation of bumble bee worker mass are not influenced by resource availability. It also appears that colonies benefit from producing a higher number of average sized workers and few exceptionally large workers.

Appendix C examines the association among body size, production costs, and lifetime work output. Six bumble bee colonies were used; three colonies received limited resources and three received unlimited resources. I used bomb calorimetry to determine the production costs of different sized workers. The energy content of different sized workers served as an estimate of production costs. I measured lifetime work effort using behavioral observations in which I tracked workers throughout their whole lives and recorded the tasks they performed within their colonies. I also measured the amount of pollen and nectar that foragers brought back to examine the relationship between forager body size and foraging loads brought into the colony. Lifetime work effort was used as a measure of a benefit that colonies receive for producing a certain sized worker.

Regardless of resource availability, I found no relationship between body size and lifetime work effort, suggesting that despite production costs, workers conduct about the same amount of work within their lives. However, although larger workers cost more to produce, I found that larger foragers bring in more food into colonies. Thus, my results suggest that the large variation in worker size within bumble bee colonies may arise from the energetic costs and the behavioral benefits of different sized workers.

Appendix D examines the association among body size, mass-specific metabolic rate, and lifespan. I measured metabolic rates of workers throughout their adult lifespans. I did not find a relationship between body size and mass-specific metabolic rate, but there was a negative correlation between mass-specific metabolic rate and adult lifespan of bumble bee workers. I also found that mass-specific metabolic rate generally decreases with age in workers. My results suggest that workers show metabolic ageing and that those with higher maintenance costs die sooner.

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APPENDIX A

PRIORITY RULES OF ENERGY ALLOCATION AT THE GROUP LEVEL: HOW
BUMBLE BEE COLONIES ALLOCATE ENERGY WHEN RESOURCE
AVAILABILITY VARIES

**Priority rules of energy allocation at the group level: how bumble bee colonies
allocate energy when resource availability varies**

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Abstract

1. Life history theory posits that physiological traits within an organism, such as growth and reproduction, compete with each other when resources are limited. Priority rules may dictate how energy is allocated among the physiological traits within an organism in relation to the amount of energy that it consumes. Much work on priority rules has been conducted at the whole organism level, but little has been conducted at the group level.
2. Does a unit of integrated individuals show priority rules of energy allocation? We used a eusocial insect, the bumble bee (*Bombus impatiens*) as our study organism to answer this question. One of the unique aspects of eusociality is that there is a reproductive division of labor within colonies, where workers are non-reproducing females who are essentially the somatic cells of their colony, while the reproductives (queens and males) are the gametic cells.
3. We subjected colonies to receive either limited or unlimited access to food resources. We then compared relative allocation towards growth and reproduction within colonies. We quantified colony allocation towards growth as the cumulative biomass of all the workers produced within colonies, and the allocation towards reproduction as the cumulative biomass of all the reproductives (males and queens) produced within colonies.
4. We found that when resources were limited, colonies allocated relatively more of their resources towards growth (cumulative worker biomass), whereas colonies with unlimited resources allocated relatively more of their resources towards reproduction (cumulative biomass of males and virgin queens).

5. The cumulative worker biomass *among* colonies was not affected by our resource availability treatment, but the biomass of the reproductives among colonies was higher on unlimited resources.
6. Growth rates of the cumulative worker biomass were marginally faster on unlimited resources, allowing colonies to initiate reproduction earlier. Growth rates of the cumulative reproductive biomass were significantly faster with unlimited resources.
7. In summary, we found that similar to whole organisms, there are group-level priority rules for *B. impatiens* colonies, and these rules may allow colonies to adjust their growth and reproduction in response to variation in resource availability.

Introduction

Life history theory is an evolutionary framework used to understand how natural selection shapes an organism's development, reproduction, and death (Stearns 1992; Roff 1992 ; Nylin & Gotthard 1998b). This theory assumes that resources are limited and this would consequently constrain life-history traits such as maintenance, growth, and reproduction (Stearns 1992; Roff 1992) as the partition of energy allocation amongst these traits may be unequal. For example, allocating more energy towards growth may hinder future allocation towards reproduction. In order to maximize energy use and fitness in a given environment, organisms must make crucial decisions of energy allocation towards various life history traits.

How should organisms allocate energy between growth and reproduction when nutrient input is low versus when nutrient input is high? Priority rules determine the

relative allocation of energy among physiological processes depending on the amount of energy input (Jokela & Mutikainen 1995a; Zera & Harshman 2001a). Such plasticity in energy allocation should enable organisms to maximize their fitness in their particular environments. Much work on priority rules has been conducted mostly at the whole-organism level (Zera & Harshman 2001a). However, life history theory could also be used to understand life history traits of eusocial insect colonies (Asano & Cassill 2011; Shik *et al.* 2012). Although a colony is composed of multiple individuals who may have conflicting reproductive interests (Heinrich 1979; Johnson *et al.* 2009), a colony functions as a whole unit physiologically and growth and reproduction can be quantified at the colony-level (Hölldobler & Wilson 2009a). Thus, the concept of priority rules should apply not only to whole organisms, but to eusocial insect colonies as well. The focus of this study is to identify the priority rules that determine how energy is allocated between growth and reproduction in eusocial insect colonies. The existence of priority rules would indicate that colonies exhibit phenotypic plasticity in energy allocation between growth and reproduction (Zera & Harshman 2001a). Such plasticity would allow colonies to cope with variation in ecological conditions.

Eusocial insect colonies are highly social and are prime examples of complex biological systems that are so integrated that they are often referred to as superorganisms. Examples include, but are not limited to, ants, bees, wasps (hymenopterans) and termites (isopterans) (Plowes 2010). By definition, a superorganism is an organized society with parts analogous to the physiological features of individual organisms (Wheeler 1911; Hölldobler & Wilson 2009a). Colonies show life cycle phases such as growth, reproduction (Hölldobler & Wilson 2009a), and senescence (Goulson 2003b), similar to

whole organisms. Colonies also show thermoregulation, just as individual organisms, where they exhibit graded responses to changes in temperatures (Weidenmüller, Kleineidam & Tautz 2002; Jones *et al.* 2004; Duong & Dornhaus 2012). Additionally, colonies show ‘cellular differentiation’ such that queens and males are analogous to gametic cells and non-reproducing female workers are analogous to somatic cells. As their name implies, workers conduct colony tasks that contribute towards growth and reproduction, such as foraging, feeding larvae, colony defense, etc. The reproductive division of labor is characteristic of eusocial insect colonies, as is the overlap of generations living in the nest and cooperative brood care (Hölldobler & Wilson 2009a). However, the reproductive division of labor is not always clear-cut because workers, who are usually non-reproducing, may sometimes attempt to lay their own eggs (haploid and unfertilized) or may rear more sisters than brothers (due to haplodiploidy, hymenopteran sisters may share up to an average of 75% of their genes and brothers may share up to 50% of their genes, if the colony has only one queen) (Bourke 1999). However, social insect colonies have methods to reduce such reproductive conflicts (e.g. queen pheromone that suppresses worker reproduction (Butler & Johnston 1962; Holman *et al.* 2010)) and promote colony cohesion.

Previous studies on whole organisms have shown that major life history traits trade-off with reproduction under certain resource conditions. For instance, reproduction overrides allocation towards growth if resources are limited, as has been suggested for the freshwater clam *Anodonta piscinalis* (Jokela & Mutikainen 1995a) and the cricket *Gryllus assimilis* (Zera, Potts & Kobus 1998). Furthermore, allocation to maintenance may be higher than allocation to reproduction when resources are limited (Boggs & Ross

1993 ; Jokela & Mutikainen 1995a). Such trade-offs with reproduction would occur because of costs due to a decreased capability to reproduce again in the future or a reduction in survival (Stearns 1989; Reznick, Nunney & Tessier 2000a).

While some eusocial insect colonies, such as perennial honey bees or harvester ants, may store enough food to last through winter or resource-poor seasons, other eusocial insects do not have such long-term stores. For instance, the annual bumble bees, such as *Bombus impatiens*, store just enough food to last for a very short period of time, such as overnight when foraging has stopped (Cartar & Dill 1991; Schmid-Hempel & Schmid-Hempel 1998; Goulson 2003b) or on cold or rainy days (Schmid-Hempel & Schmid-Hempel 1998). Because of their minimal food stores and the fact that they live for only a single flowering season (Heinrich 1979; Goulson 2003b), which may last a few to several months, the availability of food may have a substantial impact on the survival and fitness of bumble bee colonies. This is especially true for bumble bee colonies that establish nests in underground holes, remain there for one growing season, and do not move to habitats with more abundant energy resources.

Although there has been some research on how resource availability affects overall colony production of workers and/or reproductives of ants (Herbers & Banschbach 1999b; Aron, Keller & Passera 2001b; Smith 2007b), and bumble bees (Pelletier & McNeil 2003), we know little of how resource availability affects the relative investments *between* workers (colony growth) and reproductives (colony reproduction) *within* social insect colonies (but see Aron et al. 2001; Smith, 2007), especially for annual colonies that do not have long-term food stores. Knowing what the priority rules are for

resource allocation in colonies would help us understand how colonies ‘strategize’ in their habitats.

Colony allocation towards growth could be measured as the total biomass of workers produced; similarly, allocation towards reproduction could be measured as the total biomass of both males and queens that colonies produce. With this in mind, we asked the following questions in our study: 1) how does resource availability affect energy allocation between colony growth (worker production) and colony reproduction (queens and males) *within* colonies, and 2) what are the consequences of resource availability on colony-level fitness? We manipulated food availability and measured energy allocation towards growth and reproduction in an annual social insect colony, *Bombus impatiens*. Because a *B. impatiens* colony reaches the reproduction phase once in its lifetime, we predicted that colonies under limited resource conditions should allocate relatively more of their energy towards reproduction and should therefore have relatively higher cumulative reproductive biomass than cumulative worker biomass (growth) within colonies. Because an unlimited amount of energy should minimize inequality in resource allocation among life history traits, we also predicted that colonies under unlimited resource conditions should have equal allocation towards growth and reproduction, and should thus have equal cumulative reproductive and worker biomass. By testing predictions that stem from life history theory (Zera & Harshman 2001a), we could better understand if priority rules of energy allocations function at a group level, despite the potential conflicting reproductive interests amongst individuals within a group.

Methods

Study species

B. impatiens colonies are annual and can live for several months under laboratory conditions (personal observation), allowing us to keep complete track of colony growth and reproduction, from initiation to senescence. *B. impatiens* queens are usually singly mated (Schmid-Hempel & Schmid-Hempel 2000a) and like all hymenoptera, bumble bees are also haplodiploid. Thus, workers within each colony are highly related (average coefficient of relatedness (r) = .75), which should reduce queen-worker conflicts that would affect colony allocation towards growth or reproduction (Johnson *et al.* 2009). A colony is founded by a mated queen after she emerges from overwintering diapause, and she rears the first set of workers by herself. After the first set of workers emerges, they help the queen rear the rest of the colony's population. Thus, a single colony begins with one individual and expands in population size throughout the growing season. All colonies go through a growth phase in which only female workers are produced. The growth phase is followed by a reproductive phase in which worker production slows down significantly and eventually halts while virgin queens (larger females) and males are produced. Queens and males would then leave the nest to mate; their natal colonies would senesce and eventually die. Tropical bumble bee colonies, in contrast, may live for several seasons. Mated queens would then overwinter and found their own colonies the following spring (Heinrich 1979; Goulson 2003b).

Experimental methods

Six colonies of *Bombus impatiens* were purchased from Koppert Biological Systems (Romulus, MI, USA) in August 2011, and set up in a greenhouse located at the University of Arizona in Tucson, Arizona. Upon arrival, all six colonies were set up in

clear plastic nest boxes (10 cm tall x 18 cm wide x 29 cm long), covered with a Plexiglass top. Colonies were set up such that each had 1 queen, about 12 workers, and about 10 food cells. This simulated conditions similar to that near the founding phase and in the very beginning of the colony growth. No brood were included in the colonies at set up to ensure that any individuals that emerge from these colonies were indeed affected by the food treatment. For the first 5 days, colonies were given nectar solution (50% volume of Bee Happy, 50% volume of water) and pollen (Koppert Biological Systems, Romulus, MI, USA) *ad libitum* in their colonies to allow for recovery from the shipping process and successful acclimation to their new colony setup in the greenhouse. Because eggs take about 5 days to hatch (Goulson 2003b), there should not be any larvae exposed to food treatments in these first 5 days of acclimation.

After the fifth day, colonies were randomly assigned to one of two resource availability treatments: 'unlimited' and 'limited'. Three colonies were allotted per food treatment. In the unlimited treatment, colonies were given access to pollen and nectar solution 24 hours a day, whereas colonies in the limited food treatment were given access to pollen and nectar solution for 5 hours per day. The 24 hours of resource availability in the unlimited resource treatment ensured that every forager had access to food during foraging times, which occurred during daylight hours. Daylight ranged from about 10-12 hours throughout the whole experiment. Thus, foraging time for workers in the limited resource availability treatment was about 42%-50% of the foraging time that workers in the unlimited resource availability treatment had. All colonies had access to their food via a foraging arena, which was a netted cage (0.61 m wide x 0.91 m high x 8.2 m long) connected to the colony entrances. Pollen and nectar solutions were placed on a platform

(~ 0.46 m tall) at the very end of the foraging arena; two blue and yellow artificial flowers were placed near the food platform in each foraging arena as visual cues to encourage successful detection of food by the bees. Strips of yellow paper were also scattered throughout the foraging arena to provide visual cues to aid in flight during foraging. Access to food was denied in the limited food treatment simply by removing food from the foraging arena. The masses of pollen and nectar solution that colonies consumed were measured at least every other day. The experiment ran for 100 days, counting from the first day of colony set up.

Colonies were checked daily for newly emerged bees, which are distinctly white-grey in color. We measured the body mass of newly emerged bees and then marked them with color number tags using super glue to ensure that the same bees were not measured more than once. After tagging, bees were returned to their colonies if they were workers; however, virgin queens and males were immediately frozen after their body sizes were measured. Queens were easily identifiable because they were noticeably bigger and emerged later in the colony cycle, and males had noticeable 'mustaches' on their faces. The reproductives were removed to avoid problems of mating and queen-queen competition that could affect our results. Prior to measuring body mass of all the newly emerged bees, individuals were chilled in a refrigerator for about 10-15 minutes; this was done so that individuals were not moving on the scale during mass measurements. Individuals' abdomens were also squeezed so that any excreta or honey in the crops were not included in the mass measurements. Measuring only newly emerged bees controlled for age effects on body mass.

Viewing the colony as a superorganism, we considered a colony's allocation towards growth or reproduction as the cumulative biomass for all workers and reproductives, respectively. Thus, when analyzing our data, we will refer to cumulative biomass, or the biomass of all the workers or reproductives the colony produced throughout the experiment. Growth rates were measured as the weekly cumulative biomass of workers or reproductives (both males and virgin queens). We also considered the effects of resource availability treatment on the number of individuals that colonies produced.

Unless specified, Mixed Model ANOVA tests were conducted to analyze the effects of the resource availability treatment on a number of response variables; in these cases, the fixed effect was the 'resource availability treatment' and the random variable was 'colony'. All P values are given for two-tailed tests. Average values are reported as mean \pm SE unless otherwise stated. All analyses for growth rates were Mixed Model Regression tests, where the random effect was 'colony' and the fixed effects were 'weeks' and 'resource availability treatment'.

Results

Effect of resource availability on colony food consumption

Within the 100 days of the experiment, colonies with unlimited resources consumed significantly more pollen ($F_{1,4} = 9.69$; $P = 0.036$) and more nectar ($F_{1,4} = 12.68$; $P = 0.024$) than colonies with limited resources. Thus, our manipulation of resource availability affected colony consumption of resources.

Effect of resource availability on production of workers

We were unable to detect significant effects of resource availability on the number of workers produced throughout the experiment ($F_{1,4} = 0.56$; $P = 0.49$ (Fig. 1A), nor did the treatments have a significant effect on the cumulative biomass of workers produced by the end of the experiment ($F_{1,4} = 2.14$; $P = 0.22$) (Fig. 2A). However, food availability did affect the average mass of individual workers that colonies produced ($F_{1,3.9} = 23.62$; $P = 0.0087$) (Fig. 3A), where workers with unlimited resources were heavier than those with limited resources by about 28% or 0.031 grams (LS Means Student's t test; 95% CI: 0.013 to 0.049 grams).

Effect of resource availability on reproductives

Resource availability had a significant effect on the total number of reproductives (males and queens) that colonies produced by the end of the experiment ($F_{1,4} = 18.57$; $P = 0.013$) (Fig. 1B); colonies with unlimited resources produced about 122 more reproductives (LS Means Student's t test; 95% CI: 43.40 to 200.60 bees), about four times more than colonies with limited resources. More specifically, colonies with unlimited resources produced a higher number of both males ($F_{1,4} = 8.21$; $P = 0.046$) and queens ($F_{1,4} = 56.93$; $P = 0.0017$) than did the limited resource colonies.

Resource availability had a significant treatment effect on reproductive biomass ($F_{1,4} = 66.38$; $P = 0.0012$); colonies with unlimited resources had significantly higher reproductive biomass than those with limited resources. On average, about 34.9 more grams of reproductive biomass (LS Means Student's t test; 95% CI = 23.01 to 46.8 grams), (about six times more), were produced by colonies with unlimited resources than those with limited resources. Interestingly, there was no significant effect of resource

availability on the total biomass of males that colonies produced ($F_{1,4} = 2.28$; $P = 0.21$) (Fig. 2B). However, there was a significant effect on the total biomass of queens ($F_{1,4} = 105.86$; $P = 0.00050$) (Fig. 2B), where colonies with unlimited resources produced four times, or 21.59 grams (LS Means Student's t test; 95% CI = 15.76 to 27.41 grams), more in cumulative queen biomass than those with limited resources.

In terms of average mass of the reproductives, resource availability had significant effects on the mass of queens that colonies produced ($F_{1,5.97} = 11.12$; $P = 0.016$) (Fig. 3B), where individual queens were about 25% (0.13 grams) heavier, on average, with unlimited resources (LS Means Student's t test; 95% CI: 0.034 to 0.22 grams). In contrast, there were no effects on the average male mass ($F_{1,2.36} = 3.073$; $P = 0.20$) (Fig. 3C).

Effects of resource availability on the total biomass of workers vs. reproductives

To analyze whether resource availability affected colonies' allocations between workers and reproductives, we tested whether the biomasses between reproductives and workers were proportionately similar. There was a significant treatment effect on the difference between the reproductive and worker proportions (One-Way ANOVA: $F_{1,4} = 28.09$; $P = 0.0061$) (Fig. 4). On average, the cumulative reproductive biomass was proportionally higher than the cumulative worker biomass with access to unlimited resources by $0.89 \pm 8.3\%$, whereas the cumulative reproductive biomass was proportionately less than the cumulative worker biomass in the limited resource treatment by $62.0 \pm 8.3\%$. Thus, colonies with unlimited resources had relatively more *reproductive*

biomass than worker biomass whereas colonies with limited resources had relatively more *worker* biomass than reproductive biomass.

Resource availability also had a significant effect on the difference between absolute (not proportional) cumulative biomasses between reproductives and workers (One-Way ANOVA: $F_{1,4} = 37.78$; $P = 0.0036$). The average difference between the cumulative reproductive and worker biomass in colonies with unlimited resources was 19.21 ± 3.11 grams, whereas on average, the worker biomass in colonies with limited resources was more than the reproductive biomass by 7.81 ± 3.11 grams. Thus, the absolute cumulative reproductive biomass was higher than the cumulative worker biomass in colonies with unlimited resources, whereas the converse was true for colonies with limited resources.

Growth rate of cumulative biomass

The mean number of weeks that colonies produced workers was 8.67 ± 0.49 weeks. To test whether resource availability had an effect on cumulative worker biomass over time, or growth rates of total worker biomass, we analyzed whether there was a relationship between cumulative worker biomass and time from the first week of worker emergence to the fifth week. We chose the fifth week post initial worker emergence for two reasons: 1) the weekly worker biomass that colonies produced reached a maximum at or before the fifth week and 2) colonies started producing either males or queens as early as five weeks after the first workers emerged; the emergence of reproductives indicates that a colony is shifting away from its growth phase into its reproductive phase (Heinrich 1979; Goulson 2003b). Half of the colonies produced reproductives as early as five weeks and the latest was eight weeks; half of the colonies continued to have

reproductives emerging up to 12 weeks post initial worker emergence. Thus, we analyzed growth rates of reproductive biomass from weeks 5-12.

There was a marginal effect on the growth rate of cumulative worker biomass (week: $F_{4,20} = 19.64$, $P < 0.0001$; treatment: $F_{1,4} = 6.092$, $P = 0.069$) to where the growth rate seems higher when resources are unlimited (Fig. 5 A,B). In contrast, there were significant treatment effects on the growth rate of the cumulative reproductive biomass (week: $F_{7,28.46} = 9.34$, $P < 0.0001$; treatment: $F_{1,4.09} = 30.05$, $P = 0.005$) (Fig. 5 A, B). The average growth rate for reproductive biomass of colonies with limited resources was less than that for colonies with unlimited resources (LSmeans contrast: $b = -10.85$; $t_{4.092} = -5.48$; $P = 0.005$).

Discussion

We found that resource availability affects the relative energy allocations between growth and reproduction within colonies. Contrary to our predictions, colonies under unlimited resource conditions did not allocate energy equally between growth and reproduction. Rather, colonies allocated relatively more of their energy towards reproduction. We predicted that colonies under limited resource conditions would allocate relatively more energy towards reproduction; however, we found that colonies under limited resource conditions allocated relatively more energy towards colony growth. Interestingly, we found that our resource availability treatments did not significantly affect either the cumulative biomass or the number of workers that colonies produced, but both cumulative biomass and number of reproductives were significantly higher for colonies when resources were unlimited. Lastly, resource availability marginally affected colony growth rate in cumulative worker biomass and had significant

effects on cumulative reproductive biomass, where growth rates were higher when resources were unlimited than when limited.

Priority rules allow organisms to be plastic in how they dedicate nutrient input towards competing biological processes under various ecological conditions (Zera & Harshman 2001a). Our results indicate that bumble bee colonies (*B. impatiens*) have priority rules for energy allocation between growth and reproduction when faced with variation in resource availability. Based on our results, these priority rules are such that when resources are limited, colonies should invest relatively more of their internal energy resources towards growth (worker production) rather than reproduction. When resources were plentiful, however, colonies should invest relatively more of their resources towards reproduction. Similar conclusions have been drawn from other organisms such as the lepidopteran *Speyeria mormonia* (Boggs & Ross 1993) and the cotton rat *Sigmodon hispidus* (Rogowitz 1996), but are contrary to that of other organisms such as the freshwater clam *Anodonta piscinalis* (Jokela & Mutikainen 1995a).

Previous studies have shown that food supplementation affects worker population size and colony-level fitness in social insects. In ants, food supplementation increases colony reproduction (Herbers & Banschbach 1999b; Aron, Keller & Passera 2001b). Resource poor conditions led to fewer and smaller workers and reproductives in colonies of *B. terricola* (Sutcliffe & Plowright 1988a) and *B. terrestris* (Schmid-Hempel & Schmid-Hempel 1998) while field supplementation of pollen and nectar led to increased numbers of workers and reproductives in *B. impatiens* and *B. ternarius* (Pelletier & McNeil 2003). These previously mentioned studies show that limited resources would lead to overall fewer and smaller individuals in colonies and that more resources would

produce more individuals and increase colony fitness. From a life history framework, however, what is more telling is how resource availability affects the relative allocation of energy between growth (workers) and reproduction (males and virgin queens) within colonies because this would reveal how colonies cope with a given amount of energy input.

Smith (2007b) showed that in the perennial ant *Pogonomyrmex badius*, resource limitation led colonies to reallocate their energy away from reproduction and towards worker production (growth). This result matched results of long-lived organisms that can switch between growth and reproduction. The bumble bees (*B. impatiens*) we used in our study are annual colonies that do not keep long-term food stores, and they also do not switch between growth and reproduction. Because colonies are annual and must reach their reproduction phase in time before the flowering season ends, we predicted that colonies with limited resources would allocate relatively more energy towards reproduction than growth. However, we found the opposite result. This may suggest that when resources are low, the higher priority is for colonies to reach their threshold size of workers so that they can successfully rear some reproductives.

Because eusocial insect colonies are viewed as superorganisms, we used the cumulative biomass of individuals that colonies produced to understand how energy input is translated into energetic investment towards colony growth and colony reproduction. We also acknowledge that the number of individuals produced by colonies is important, especially with reference to the reproductives (i.e. virgin queens and males). While the number of individuals is important to consider, the quality, as measured by body mass, should be considered as well. It is possible that quality/quantity trade-offs may occur

under stressful conditions (Sinervo 1990; Roff 1992 ; Doughty & Shine 1997). For instance, it may very well be possible that a colony produces less queens, but those queens could be of higher quality. Here, we assume that a heavier individual could be of higher quality because heavier individuals may contain more absolute muscle mass and lipid stores.

We did not find any evidence for quantity/quality trade-offs of workers in our study. Our results show that both the number and cumulative biomass of workers were statistically similar between the limited and unlimited food treatments. This is contrary to previous studies of food manipulation in bumble bees (Schmid-Hempel & Schmid-Hempel 1998; Pelletier & McNeil 2003) in which more food resources led to a higher number of workers produced by colonies. It is possible that a larger sample size would have given us statistical significance. However, there were slight trends in our results to where both the number and cumulative biomasses was slightly higher overall when resources were unlimited. This trend coincides with our finding that the average mass of an individual worker was significantly higher in the unlimited treatment, suggesting that workers may be of higher quality when resources are unlimited.

Similarly, we did not find any evidence for quantity/quality trade-offs of reproductives in our study. We found that both the number of reproductives (males + queens) and the cumulative reproductive biomass were higher with unlimited resources, and this is because of the higher number and cumulative biomass of the queens. The average mass of an individual queen was also higher in the unlimited treatment. The number of males was higher when resources were unlimited, but the average mass of an individual male was not affected by the resource availability treatment. There were also

no treatment effects on the cumulative biomass of males that colonies produced. This suggests that there are no quantity/quality trade-offs in male or queen production.

Heavier queens have more lipid stores (Richards 1946a; Cumber 1949). Because mated queens enter diapause during the winter and found colonies on their own (Heinrich 1979; Goulson 2003b), more lipid stores should increase colony founding success (Beekman, van Stratum & Lingeman 1998). Results from our study suggest that when given ample resources, colonies produce both higher quantity and higher quality of virgin queens that would have higher chances of surviving winter and founding future colonies.

Although statistically not significant ($P = 0.069$), there was a trend to where colony growth rates in the cumulative worker biomass were faster when resources were unlimited. Thus, the rate to reach a certain 'size' of total worker biomass was faster when resources were unlimited. Considering that the cumulative worker biomass between the limited and unlimited resource availability treatments were similar, this may indicate that there may be a threshold size that colonies must reach in order to successfully rear reproductives. It is anecdotally stated in the literature that it is actually colony density and worker to larvae ratio that may trigger reproduction in colonies (Goulson 2003b), but whether or not this is true is still unknown. Given that all colonies were settled in nest boxes of the same sizes in our study, it is possible that colony density triggers reproduction. Nevertheless, whether it is colony maturation size or maturation density, colonies that receive unlimited resources may reach the growth end-point faster, and hence, begin reproduction earlier. Similarly, growth rates of cumulative reproductive biomass were faster when resources were unlimited.

One caveat in this study is that our manipulation of resource availability was such that colonies had a constant exposure to their assigned treatment type (limited vs. unlimited access to food) and more likely simulates spatial variation in resource availability. However, under natural conditions, it is likely that colonies experience temporal variation in resource availability throughout the flowering season. We hypothesize that bumble bee colonies' responses to temporal variations in resources would be plastic such that they could adjust energy allocation towards growth or reproduction as needed. For example, if a colony that was experiencing a time of plentiful resources suddenly experiences a condition of less resource availability because of a sudden storm or drought, colonies may respond by investing less of their resources towards reproductives and more towards workers. Furthermore, it is important to note that we used biomass as a measure of energy allocation. Technically, energy allocation should be measured in energy units, such as joules or calories. However, there is evidence that mass would be a good estimator of energy allocation, as has been supported for *Gryllus* crickets (Zera & Harshman 2001a). We also have preliminary evidence that there is a positive correlation between worker bumble bee (*B. impatiens*) body mass and calories (unpublished data).

Growth, reproduction, storage, and senescence are traits that are easily identifiable and quantifiable without harmful disturbance to a eusocial insect colony. Thus, we propose that eusocial insect colonies are useful models for testing hypotheses of life history theory and dynamic energy budgets to investigate how environmental stress affects energy allocation towards traits at the group-level. To our knowledge, our study is

one of the few that has studied group-level energy allocation between growth and reproduction within colonies as a response to resource availability.

Overall, when resource availability differs, bumble bee colonies (*B. impatiens*) have priority rules of energy allocation between growth and reproduction. When resources are unlimited, colonies should prioritize energy input towards reproduction, whereas when resources are limited, colonies should prioritize energy input towards growth. Despite possible reproductive conflicts that could have arisen in the colony, there were consistent emergent patterns at the group level for plasticity in energy allocation towards growth and reproduction. These priority rules affect colony fitness and are strategies that enable colonies to cope with variation in resource availability.

Acknowledgements

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Figures & Captions

Figure 1.

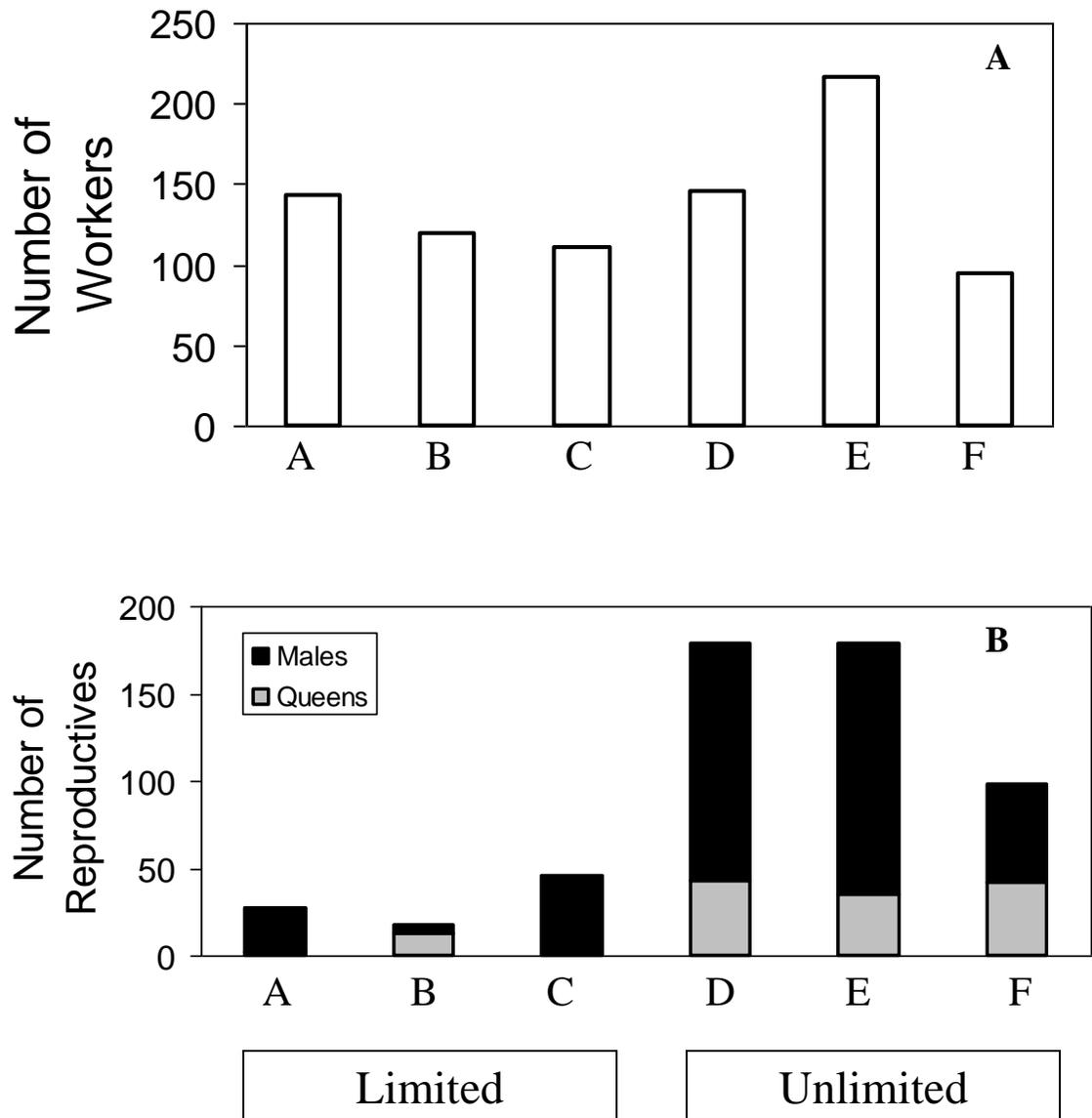


Figure 1. This figure shows the effects of resource availability treatment (limited and unlimited) on the total number of workers (panel A) and reproductives (panel B) that colonies produced. Colonies A, B, and C were in the limited resource availability treatment, and colonies D, E, and F were in the unlimited treatment. Resource availability treatment did not have significant effects on the total number of workers produced (panel A). However, more reproductives were produced in the unlimited treatment. Both males

and queens were produced at significantly higher numbers in the unlimited treatment (panel B).

Figure 2.

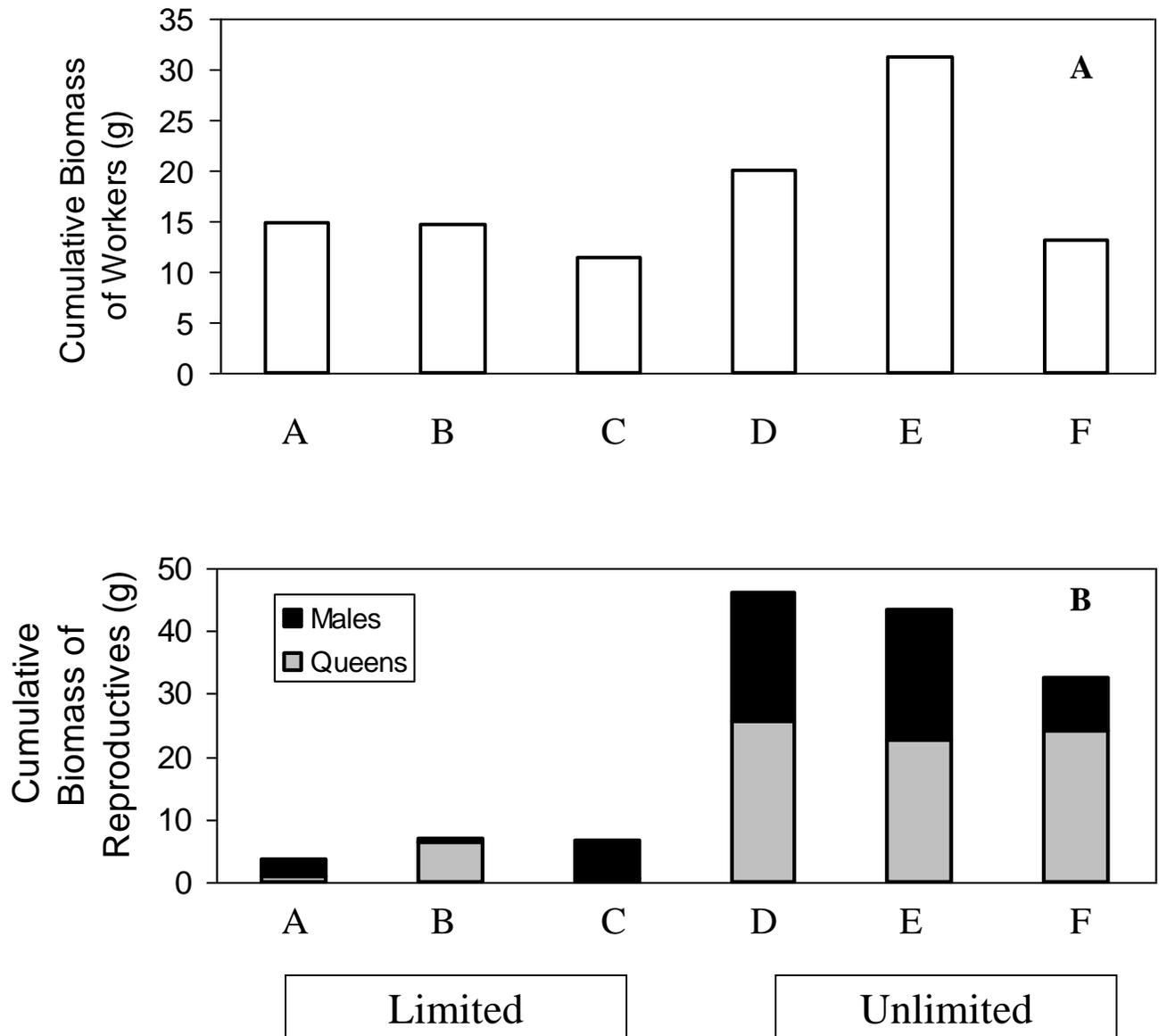


Figure 2. This figure shows the effects of resource availability treatment on the cumulative biomass of workers (A) and reproductives (B) that colonies produced. There were no significant effects of resource treatment on the cumulative biomass of workers (panel A). However, colonies (A, B, and C) in the unlimited resource availability treatment had a higher reproductive biomass than those in the limited resource treatment (colonies D, E, and F) (panel B). Colonies in the unlimited treatment produced significantly more queen biomass than those in the limited treatment, but cumulative male biomass was similar between the two resource treatments.

Figure 3.

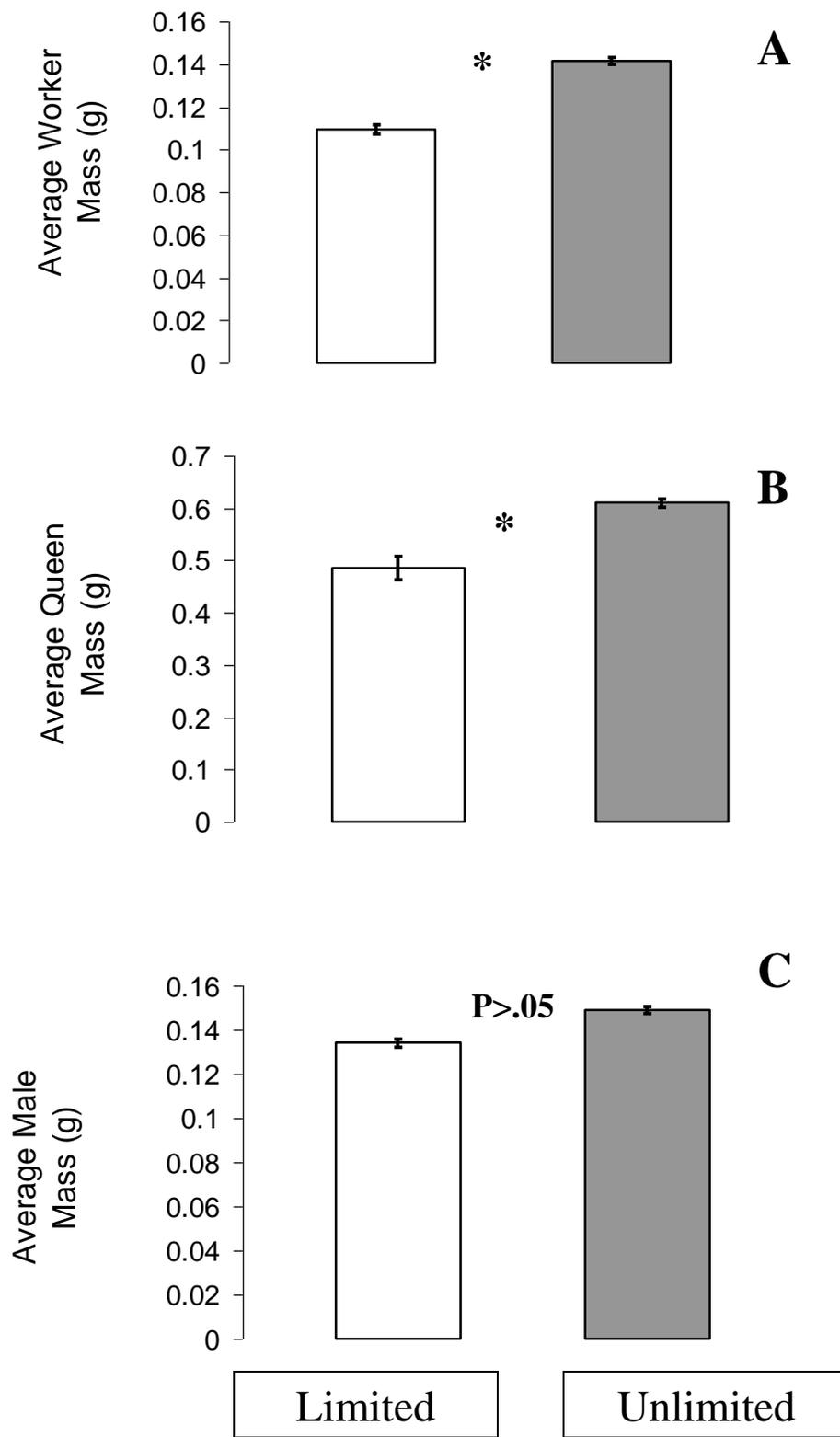


Figure 3. The effects of resource availability on the average mass \pm SE of workers (A), queens (B), and males (C). * indicates $P < .05$

Figure 4.

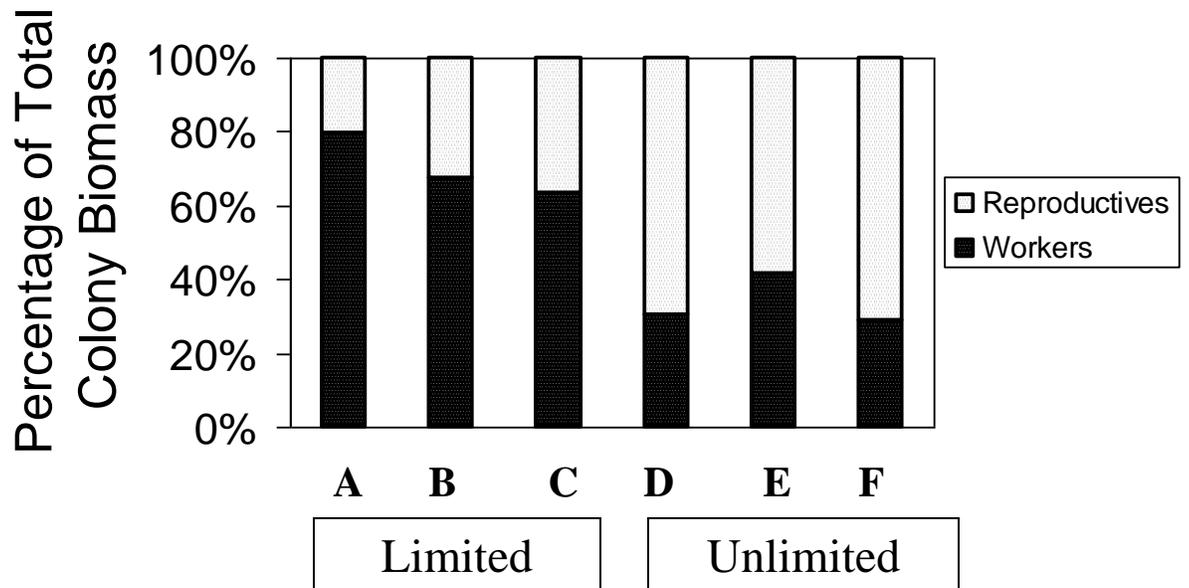


Figure 4. The effects of resource availability treatment on the relative percentages of the total biomass within colonies that are composed of workers vs. reproductives (males + virgin queens). Colonies in the limited resource treatment (A-C) had relatively more worker biomass. Conversely, there was relatively more reproductive biomass for colonies D-F in the unlimited resource availability treatment.

Figure 5.

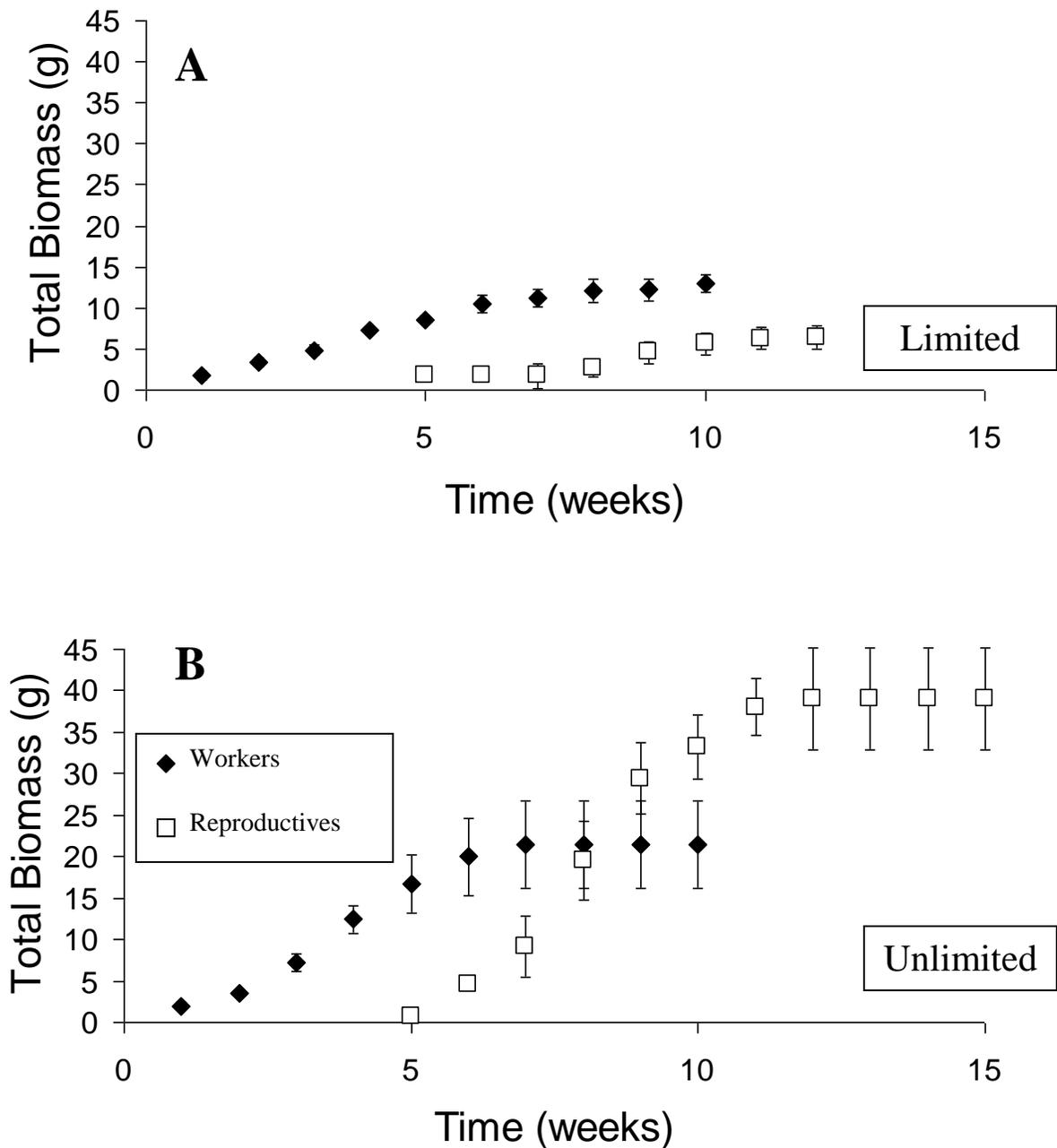


Figure 5. This figure shows the absolute values for the cumulative biomasses of workers and reproductives (queens + males) for colonies in the limited (A) and unlimited (B) resource availability treatments. The time sequence (in weeks) starts from the first week that the first workers emerged in the experiment. Each shape represents the average total biomass \pm SE, as averaged across three colonies per treatment. Growth rates for cumulative worker biomass in the first five weeks were marginally faster in the unlimited

treatment. In addition, growth rates for the cumulative reproductive biomasses from weeks 8-12 were significantly faster in the unlimited treatment.

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EFFECTS OF RESOURCE AVAILABILITY ON WORKER SIZE DISTRIBUTION IN
BUMBLE BEE (*BOMBUS IMPATIENS*) COLONIES

Effects of resource availability on worker size distribution in bumble bee (*Bombus impatiens*) colonies

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Summary

There are benefits and costs to being of a certain body size, and this gives rise to size variation amongst individuals. For social insects such as ants, termites, and bumble bees, body size variation is an important group-level trait because for these insects, morphology plays a role in division of labor with certain sizes typically performing certain tasks. Task efficiency is thought to be the reason for the association between body size and task performance. In bumble bees for instance, larger workers are more efficient at collecting food, and thus, are the ones who leave the nest and forage while smaller workers perform tasks within the nest. Because amount of resources received during larval development affects adult body size, worker size distribution can be viewed as differential costs of worker production, where smaller individuals are cheaper than larger individuals. Given a limited amount of resources, colonies could produce many small individuals, a mixture of different sized individuals, or few large individuals. Because the distribution of worker size may impact colony fitness, we investigated the effects of resource availability on worker size distribution in bumble bee (*B. impatiens*) colonies. We were also interested in how size distribution may develop throughout the colony growth phase. We subjected colonies to receive either limited or unlimited resources and measured the body masses of newly emerged workers. One highlight of our study is that colonies were reared from the very beginning of colony growth and data were collected until the very end of colony growth. We found that resource availability did not affect the overall distribution of worker mass or standard deviation, our measure of size variation. For all workers that colonies produced, size distributions were continuous but not normally distributed; distributions were positively skewed and most had platykurtic

peaks. Worker size was not always normally distributed throughout colony growth; those that were not normal were usually platykurtic and positively skewed. Skew, kurtosis, and standard deviation of worker mass were not significantly affected by colony growth period or resource availability. However, worker mass at the end of colony growth was higher than at the beginning of colony growth, regardless of resource availability. From our data, we conclude that the shape and variation of bumble bee worker mass are not influenced by resource availability. It also appears that colonies benefit from producing a higher number of average sized workers and few exceptionally large workers.

Introduction

Body size is generally a strong predictor of organismal fitness and is thus an important life history trait (Roff 1992b; Stearns 1992). For example, larger odonate males have higher mating rates and lifetime mating success than smaller males (Sokolovska, Rowe & Johansson 2000) and there is a positive correlation between female body mass and fecundity in most insect taxa (Honěk 1993). There are also advantages to being small, such as smaller Dipteran males being more agile and having higher mating success (McLachlan & Allen 1987; Steele & Partridge 1988) or smaller worker bumble bees (*Bombus impatiens*) surviving starvation longer than their larger sister bees (Couvillon & Dornhaus 2010). Less provisional resources would also be required to produce smaller individuals (Bosch & Narcís 2002). As a result of both the benefits and costs of different body sizes, size variation exists in bumble bee colonies.

The benefits associated with different body sizes make size variation, and size distribution in general, important life history traits for some social insects such as ants (Wilson 1980; Wood & Tschinkel 1981; Hölldobler & Wilson 1990), termites (Miura &

Matsumoto 1995), and bumble bees (Goulson *et al.* 2002; Goulson 2003a; Jandt & Dornhaus 2009a; Jandt, Huang & Dornhaus 2009a; Couvillon *et al.* 2010). A social insect colony functions as a whole unit and consists of individuals from different castes: reproductive queens and males and non-reproductive workers. Although non-reproductive workers generally have little to no direct fitness, they can contribute towards their inclusive fitness by working in the colony, caring for and rearing related reproductives (Hamilton 1964).

In a division of labor amongst workers, tasks are organized primarily either by age, body size (Beshers & Fewell 201), or dominance (West-Eberhard 1969). Concomitantly with a morphological division of labor, there is large intra-colony size variation among workers (Alford 1975; Wilson 1980; Hölldobler & Wilson 1990; Goulson *et al.* 2002; Araujo & Tschinkel 2010), more so than in other social insects. For instance, workers can vary up to 500 fold in body mass in the Asian marauder ant, *Pheidologeton diversus* (Hölldobler & Wilson 1990).

Bumble bee (*Bombus* spp.) workers can vary up to 10 fold in mass, which is about five times more than in other bees (Waddington, Herbst & Roubik 1986; Ramalho, Imperatriz-Fonseca & Giannini 1998; Roulston & Cane 2000). Bumble bee queens are usually singly mated (Schmid-Hempel & Schmid-Hempel 2000a) and workers reared within a colony are highly related sisters ($r = 0.75$). Division of labor is related to body size in which larger workers tend to forage, guard, and fan while smaller workers tend to incubate and feed larvae within the nest (Richards 1946b; Goulson *et al.* 2002; Jandt & Dornhaus 2009a).

Body size for a worker bumble bee is positively correlated with the amount of food it receives during larval development (Plowright & Jay 1968; Pendrel & Plowright 1981; Sutcliffe & Plowright 1988a; Sutcliffe & Plowright 1990; Pereboom, Velthuis & Duchateau 2003). Larvae feed on pollen at the beginning of larval development, but are then later fed a mixture of pollen, nectar, and a mixture of proteins secreted from adult workers. The ratio of pollen, protein, and carbohydrates are similar for all castes (Pereboom 2000). In addition, there is a positive correlation between worker body mass and energy content (Cao and Davidowitz, in prep), further indicating that larger workers cost more energy resources to produce. A proximate mechanism for the production of bumble bee worker (*B. impatiens*) size variation is related to larval location within the nest; the closer larvae are to the middle of the nest, the more often it is fed by adult workers (Couvillon & Dornhaus 2009b), and hence the larger it is as an adult. Under plentiful resources, bumble bee (*B. impatiens*) worker body size is generally normally distributed as a colony ages, and worker size variation generally decreases with colony age. The size of all workers produced is also generally normally distributed within colonies (Couvillon *et al.* 2010).

A bumble bee colony is founded by a single queen, and it grows from one individual to hundreds, depending on the species. Failure rates are high (Goulson 2003a) and availability of floral resources strongly impact the success of colony survival and reproduction (Bowers 1985). Growth in social insect colonies pertains to a stage in a colony's life cycle in which workers are reared, and it halts when colonies reach the reproduction stage and rear virgin queens and males. Knowing that the amount of food

larvae receive during development affect their adult size, we can consider worker size distribution as a reflection of differential production costs.

In this study, we ask: 1) how does worker size distribution change from the very beginning of colony growth, right after a queen founds her colony and raises her first batch of workers? Would size distribution be different towards the end of colony growth, once colonies grow in population size and are able to bring in more resources? 2) how could resource limitation affect the overall worker size distribution as well its ontogeny?

In general, colonies produce smaller workers, on average, when resources are limited (Cao and Davidowitz, in prep). It is possible that when resources are limited, the size distribution for all newly emerged workers colonies produce would be skewed to the right, in which case colonies produce a higher number of smaller and cheaper workers and very few larger workers. Alternatively, overall worker size distribution could be symmetrical and normally distributed. It is also possible that regardless of the amount of resources a colony receives, the average worker size in the beginning of colony growth would be smaller than that at the end of colony growth because there are fewer workers and incoming energy resources in the very beginning of colony growth. Furthermore, if size variation is beneficial for bumble bee colonies, we expect that overall size variation would be similar in colonies with limited and unlimited resources; we also expect the size variation to be maintained throughout colony growth. Understanding the development of worker size distribution during colony growth and how resources affect worker size distribution would allow us to understand how colonies allocate energy resources towards growth.

Methods

Study system

B. impatiens colonies are annual and can live for several months under laboratory conditions (personal observation), allowing us to keep complete track of colony growth and reproduction, from initiation to senescence. A colony is founded by a mated queen after she emerges from overwintering diapause, and she rears the first set of workers by herself. After the first set of workers emerges (8-16 workers), they help the queen rear the rest of the colony's population (Goulson 2003a). Thus, a single colony begins with one individual and expands in population size throughout the growing season. All colonies go through a growth phase in which only female workers are produced. The growth phase is followed by a reproductive phase in which worker production slows down significantly and eventually halts while virgin queens (larger females) and males are produced. Queens and males would then leave the nest to mate; their natal colonies would senesce and eventually die. Tropical bumble bee colonies, in contrast, may live for several seasons. Mated queens would then overwinter and found their own colonies the following spring (Heinrich 1979; Goulson 2003a).

Experimental methods

Six colonies of *Bombus impatiens* were purchased from Koppert Biological Systems (Romulus, MI, USA) in August 2011, and set up in a greenhouse located at the University of Arizona in Tucson, Arizona. Colonies were set up in clear plastic nest boxes (10 cm tall x 18 cm wide x 29 cm long), each covered with a Plexiglas top and

included 1 queen, about 12 workers, and about 10 food cells. The 12 workers that were included in the colony set-up represented a realistic number for a colony's first batch of newly emerged workers. This simulated conditions close to the beginning of the colony growth. No brood were included in the colonies at set up to ensure that individuals who emerged from these colonies were affected by the food treatment. For the first 5 days, colonies were given nectar solution (50% volume of Bee Happy, 50% volume of water) and pollen (Koppert Biological Systems, Romulus, MI, USA) *ad libitum* in their colonies to allow for recovery from the shipping process and successful acclimation to their new colony setup in the greenhouse. Because eggs take about 5 days to hatch (Goulson 2003a), there should not be any larvae exposed to food treatments in these first 5 days of acclimation.

After the fifth day, colonies were randomly assigned to one of two resource availability treatments: 'unlimited' and 'limited'. Colonies labeled L1, L2, and L3 were in the limited resource treatment while colonies U1, U2, and U3 were in the unlimited resource treatment. Three colonies were allotted per food treatment. In the unlimited treatment, colonies were given access to pollen and nectar solution 24 hours a day, whereas colonies in the limited food treatment were given access to pollen and nectar solution for 5 hours per day. The 24 hours of resource availability in the unlimited resource treatment ensured that every forager had access to food during foraging times, which occurred during daylight hours. Daylight ranged from about 10-12 hours throughout the whole experiment. Thus, foraging time for workers in the limited resource availability treatment was about 42%-50% of the foraging time that workers in the unlimited resource availability treatment had. All colonies had access to their food in a

foraging arena, at the end of a netted flight tunnel (0.61 m wide x 0.91 m high x 8.2 m long) connected to the colony entrances. Pollen and nectar solutions were placed on a platform (~ 0.46 m tall) at the very end of the foraging arena; two blue and yellow artificial flowers were placed near the food platform in each foraging arena as visual cues to encourage successful detection of food by the bees. Strips of yellow paper were fixed to the vertical sides of the flight tunnel to provide visual cues to aid in flight during foraging. Access to food was denied in the limited food treatment simply by removing food from the foraging arena. The masses of pollen and nectar solution that colonies consumed were measured at least every other day. The experiment ran for 100 days, counting from the first day of colony set up. Colonies progressed through the growth, reproduction, and senescence stages. Since workers emerge during the growth phase and worker production ceases when colonies switch to producing reproductives, this allowed us to measure the body sizes of any workers that colonies could potentially produce throughout their lifespans.

The first workers emerged between 3 and 4 weeks after colony setup. Colonies were checked daily for newly emerged bees, which are distinctly white-grey in color. We used worker body mass as a measure of worker body size. We measured the body mass of newly emerged bees and then marked them with color number tags to ensure that the same bees were not measured more than once. Workers were immobilized by chilling for about 10-15 minutes prior to weighing. Individuals' abdomens were also squeezed so that any excreta or honey in the crops were not included in the mass measurements. Measuring only newly emerged bees controlled for age effects on body mass.

Statistical analyses

To examine the distributions of worker size, we tested for normality of worker body mass using Shapiro-Wilk tests. Size variation was measured as standard deviation. Sample skew (G1) and sample excess kurtosis (G) were calculated as values to describe the shapes of worker size distributions. A sample skew value of 0 indicates that a distribution is symmetrical. If a distribution has a positive sample skew value, it is skewed to the right, meaning the distribution has a tail that extends to the right. If sample skew is negative, the distribution is skewed to the left. A sample skew value that is greater than +1.0 or less than -1.0 indicates that the distribution is highly skewed. A normal distribution has a sample kurtosis value of 3 and it is mesokurtic. The sample excess kurtosis value can be thought of as a sample kurtosis value that has been corrected so that a normal distribution has zero kurtosis. Thus, a sample excess kurtosis value of 0 indicates that a distribution has a mesokurtic peak; a positive sample excess kurtosis value indicates a leptokurtic distribution (i.e., has a pointy peak), and a negative excess kurtosis value indicates a platykurtic distribution (i.e. broad peak). Colony growth was separated into three time periods: beginning, middle, and end. The start of colony growth starts on the first day that workers began to emerge from pupae. Thus, week 1 indicates the first week of worker emergence. The number of weeks during which workers emerged from pupae was observed to range from 7-10 weeks among all six colonies. The 'beginning' indicates weeks 1-3 of worker emergence, 'middle' indicates weeks 4-6 of worker emergence, and the 'end' of worker emergence indicates weeks 7-10 of worker emergence.

Unless otherwise stated, all means are reported as mean \pm SE. To analyze whether skew and kurtosis of worker size vary throughout colony growth and by resource

availability, a multi-way ANOVA was conducted, with colony growth period and resource availability as fixed effects, an interaction between the fixed effects, and with colony as a random factor. Sample skew values (G1) and sample excess kurtosis values (G) were calculated for worker size distributions for each colony in each growth period (beginning, middle, and end) as well as for the size distribution of all workers produced in all colonies.

Results

Size distributions of all workers produced

All colonies, regardless of resource availability treatment, had non-normal distributions for body mass of all workers produced (Shapiro-Wilk test: all $P \leq 0.0124$). Colony U2 had a highly positive skew and a leptokurtic (pointy peakedness) distribution. Colony L1 had a moderately positive skew with a platykurtic (broad and flat peakedness) distribution. The other colonies had moderately positive skew with leptokurtic distributions of worker mass (Table 1 & Fig. 1). A positive skew indicates a distribution with more small and medium sized workers and much fewer larger workers. A platykurtic distribution has a peak around the mean that is broad and flat in shape rather than ‘pointy’ as in a leptokurtic distribution. On average, the maximum worker mass was 5.4 ± 0.82 times more than the minimum worker mass within colonies (N = 6 colonies). Resource availability did not have a significant effect on the standard deviation of worker size (Mixed Model ANOVA with ‘colony’ as random effect: resource availability, $F_{1,4} = 0.080$, $P = 0.79$).

Size distributions of newly emerged workers throughout colony growth

Three of six colonies had normal distributions of mass for newly emerged workers in the beginning of the colony growth cycle, two of which received limited resources and the other received unlimited resources. Three of six colonies had normal worker mass distributions in the middle of colony growth, one of which received limited resources and the others received unlimited resources. Five of six colonies had normal worker mass distributions; the one that was not normally distributed had received limited resources (Fig. 2). The size distributions of newly emerged workers were mostly but not always normally distributed throughout colony growth and there appears to be no effect of resource availability treatment on worker size distribution throughout colony growth. When non-normally distributed, size distributions of newly emerged workers were usually platykurtic and moderately skewed to the right (Fig. 2 for example frequency distributions); in one case (Colony U1, end of colony growth period), there was a highly positive skew (Fig. 2). There were no significant colony growth period or resource availability effects on the skew of worker size. There was also no significant growth period X resource availability effect (Multi-way ANOVA with colony as random effect: colony growth period, $F_{2,8} = 0.322$, $P = 0.73$; resource availability, $F_{1,4} = 0.14$, $P = 0.73$; colony growth period x resource availability, $F_{2,8} = 0.0028$, $P = 0.99$)

Similarly, kurtosis was not significantly affected by colony growth period or resource availability. There was also no significant growth x resource availability effect (Multi-way ANOVA with colony as random effect: colony growth period, $F_{2,8} = 1.94$, $P = 0.21$; resource availability, $F_{1,4} = 1.73$, $P = 0.26$; colony growth period x resource availability, $F_{2,8} = 1.29$, $P = 0.33$).

Standard deviation of worker mass was not affected by colony growth period or resource availability. There was no significant colony growth x resource availability effect on the standard deviation of worker mass (Multi-way ANOVA with colony as random effect: colony growth period, $F_{2,8} = 0.20$ $P = 0.83$; resource availability, $F_{1,4} = 0.35$, $P = 0.59$; colony growth period x resource availability, $F_{2,8} = 1.30$, $P = 0.32$)

There were significant effects of colony growth period and resource availability on worker body mass. There was also a significant colony growth period x resource availability interaction effect on worker mass (Multi-way ANOVA with 'colony' as random effect: colony growth period, $F_{2,822.9} = 4.99$ $P = 0.0070$; resource availability, $F_{1,5.16} = 8.11$, $P = 0.035$; colony growth period x resource availability, $F_{2,822.9} = 14.48$, $P < 0.0001$). Body mass differed significantly for colonies in the limited resource treatment only between the beginning and end of colony growth; mass was higher at the end of colony growth 0.017 ± 0.0056 grams, or by 16%, when compared to the mass of workers from the beginning of colony growth. For colonies in the unlimited resource treatment, worker mass increased significantly between the beginning and middle of colony growth (LSMeans differences Tukey HSD comparisons, $P < 0.05$) (Fig. 3).

Discussion

In this study, resource availability did not affect the size variation of all the workers produced in colonies. Furthermore, we found that size distributions of all workers that colonies produced were not normally distributed, regardless of colony and resource availability treatment. The size distributions were all positively skewed and all but one colony had a leptokurtic peak, indicating that colonies produced very few exceptionally large workers and more average sized workers (Fig. 1).

In a previous study, it was found that larger bumble bee workers (*B. impatiens*) have more energy content and thus, cost more energy to produce than smaller workers. Despite this difference in production costs, workers have similar lifetime work efforts regardless of their body size (Cao and Davidowitz, in prep).; thus, smaller workers may give colonies ‘more bang for the buck’ and produce more lifetime work effort per unit of energy spent in worker production. The expensive production costs of larger workers may explain the positive skew of worker size distributions that we found in this study. Additionally, it was suggested by Cao and Davidowitz (in prep) that despite the expensive costs of larger workers, colonies benefit from producing some larger workers because they carried in larger pollen and nectar loads, and thus, more energy resources into the colony than smaller foragers.

The body mass of newly emerged workers was usually normally distributed for colonies throughout the beginning, middle, and end of colony growth. There was no consistent pattern for normal size distributions with regards to effects of resource availability and colony growth period. Size distributions for newly emerged workers that were not normally distributed were usually platykurtic with a moderately positive skew (Table 2 & Fig. 2), indicating that throughout colony growth, colonies continued to produce a low number of exceptionally large and costly workers and more average sized workers. One adaptive explanation for producing smaller workers in social insects is that smaller workers are produced in the beginning of colony growth when resources are limited so that the founding queen can produce more workers to aid in colony growth, such as in the red imported fire ants, *Solenopsis invicta* Buren (Porter & Tschinkel 1986). It is possible that the positive skews for the cumulative distributions of bumble bee

worker sizes that we found in this study reflect an advantage of producing a higher number of average sized workers than very large workers because of the costs associated with larger bees (Cao and Davidowitz, in prep).

Colony growth period and resource availability did not have significant effects on the skew or kurtosis on the distributions for body mass of newly emerged workers. Similarly, standard deviations of worker body mass did not differ significantly between resource availability treatments or throughout colony growth. Thus, regardless of whether colonies received limited or unlimited resources, colonies produced a large variation in worker size throughout the beginning, middle, and end of colony growth. One hypothesis as to why social insect colonies produce large worker size variation is that size variation is adaptive for division of labor in social insects. For example, in leaf-cutter ant workers, *Atta sexdens*, the smallest ants work within the nest, medium sized ants forage, and the largest ants are specialized for nest defending because of their relatively large mandibles (Wilson 1980). In bumble bees, larger workers are better at foraging (Goulson *et al.* 2002; Spaethe & Weidenmüller 2002). Although smaller workers survive longer under starvation conditions (Couvillon & Dornhaus 2010), not much is known about the adaptive benefits of smaller workers with relation to division of labor. It has been proposed that smaller workers are produced because they are more nimble at performing tasks within the nest (Goulson *et al.* 2002; Couvillon & Dornhaus 2010). Whether or not worker size variation is adaptive for division of labor in bumble bees is still not clear.

In our study, we also found a significant interaction between resource availability and colony growth period on the average worker body mass. When colonies received limited resources, workers that emerged during the end of colony growth were

significantly heavier than those that emerged at the beginning of colony growth. In colonies that received unlimited resources, workers that emerged during the end of colony growth were significantly lighter than those that emerged in the beginning or middle of colony growth (Fig 3). In a previous study where colonies received plentiful resources, it was found that body size of newly emerged workers, as measured by thorax width, tended to decrease over time (Couvillon *et al.* 2010). Our results for colonies that received unlimited resources are similar to these previous results. Cao and Davidowitz (unpublished data) found that colonies under unlimited resource conditions allocate relatively more energy towards producing reproductives than workers. Considering that the end of worker production signals the beginning of producing queens and males, it is possible that the average worker size decreases towards the end of colony growth because colonies prioritize reproduction over worker reproduction under unlimited resource conditions (Cao and Davidowitz, unpublished data). The increase in worker body mass that we observed through colony growth under limited resource conditions may result from colonies prioritizing energy allocation towards growth rather than reproduction (Cao and Davidowitz, unpublished data).

Here, we investigated the effects of resource availability on worker size distribution in bumble bee colonies, with the notion that the amount of resources available to a colony could affect energy allocation towards worker production and affect the cumulative worker size distribution and size distribution throughout colony growth. One highlight of our study is that colonies were set up such that they were very close to the very beginning of colony growth and every newly emerged worker that was weighed in this study was reared under its colony's assigned resource availability treatment. We

found that resource limitation does not influence the shape of bumble bee worker size distribution or the variation, indicating that perhaps these two aspects of worker size are important colony level traits under stabilizing selection.

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Figures

Table 1.

Colony	Number of workers	G1 (sample skewness)	Skew description	G (sample excess kurtosis)	Kurtosis description
L1	142	0.512	Moderately right	-0.084	Platykurtic
L2	120	1.251	Highly right	1.814	Leptokurtic
L3	111	0.874	Moderately right	0.256	Leptokurtic
U1	145	0.703	Moderately right	1.474	Leptokurtic
U2	216	1.429	Highly right	5.363	Leptokurtic
U3	95	0.689	Moderately right	0.589	Leptokurtic

Table 1. Colonies L1-L3 received limited resources while colonies U1-U3 received unlimited resources. This table shows the calculated values and descriptions for sample skew and sample excess kurtosis values of size distributions for newly emerged workers.

Figure 1.

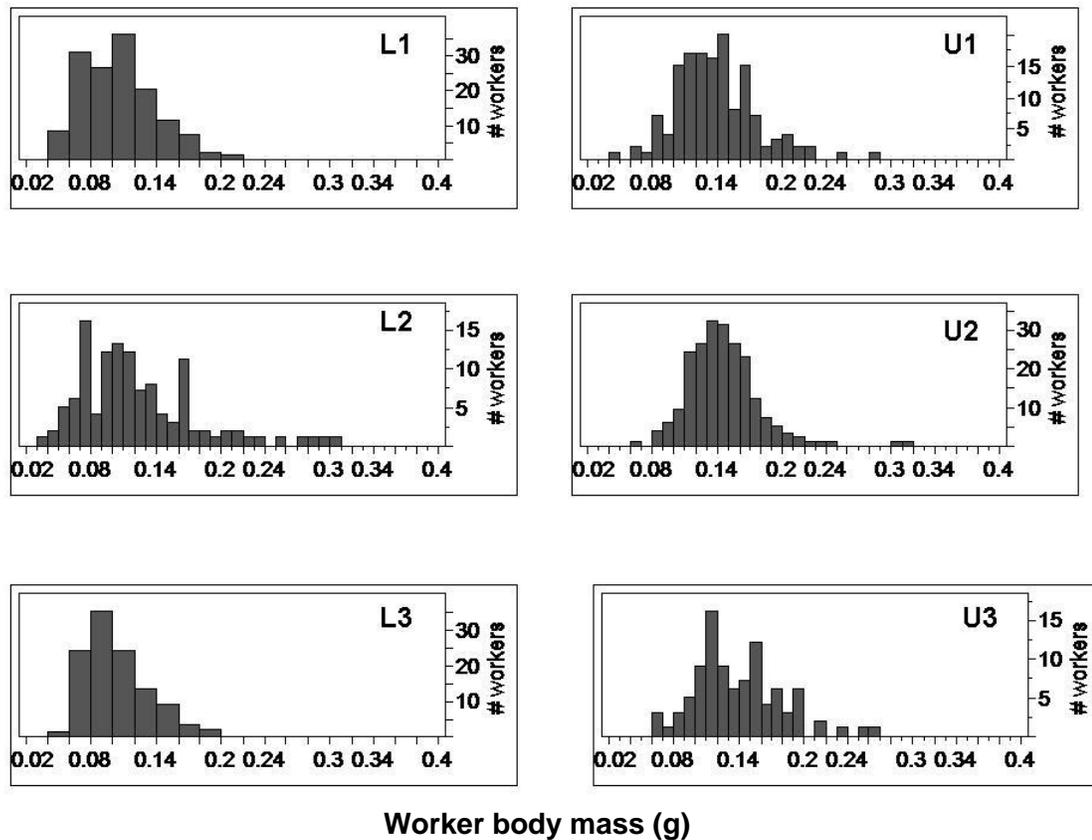


Figure 1. Frequency distributions of body mass for workers that colonies produced. Letters and numbers in each panel indicate colonies. Colonies L1-L3 received limited resources while colonies U1-U3 received unlimited resources. All distributions were not normally distributed.

Table 2.

Colony	Growth period	Resource Availability Treatment	Normally distributed?	G1 (sample skewness)	Skew description	G (sample excess kurtosis)	Kurtosis description
L1	Beginning	Limited	No, P = 0.032	0.678	Moderately right	-0.237	Platykurtic
L1	Middle	Limited	Yes, P = 0.765	0.237	≈ Symmetric	-0.141	Platykurtic
L1	End	Limited	No, P = 0.0467	0.732	Moderately right	-0.018	Platykurtic
L2	Beginning	Limited	Yes, P = 0.3364	0.321	≈ Symmetric	0.394	Leptokurtic
L2	Middle	Limited	No, P = 0.0031	0.820	Moderately right	-0.078	Platykurtic
L2	End	Limited	Yes, P = 0.0688	0.420	≈ Symmetric	-1.113	Platykurtic
L3	Beginning	Limited	Yes, P = 0.7914	0.149	≈ Symmetric	-0.452	Platykurtic
L3	Middle	Limited	No, P = 0.0029	0.591	Moderately right	-0.652	Platykurtic
L3	End	Limited	Yes, P = 0.7005	-0.600	Moderately left	-0.942	Platykurtic
U1	Beginning	Unlimited	No, P = 0.0093	0.767	Moderately positive	1.201	Leptokurtic
U1	Middle	Unlimited	Yes, P = 0.7422	0.091	≈ Symmetric	-0.177	Platykurtic
U1	End	Unlimited	Yes, P = 0.3185	1.465	Highly right	2.352	Platykurtic
U2	Beginning	Unlimited	No, P = 0.0063	0.683	Moderately right	-0.309	Platykurtic
U2	Middle	Unlimited	No, P < 0.0001	1.829	Highly right	6.826	Leptokurtic
U2	End	Unlimited	Yes, P = 0.8726	0.187	≈ Symmetric	-0.498	Platykurtic
U3	Beginning	Unlimited	Yes, P = 0.5828	0.095	≈ Symmetric	0.190	Leptokurtic
U3	Middle	Unlimited	Yes, P = 0.7562	0.130	≈ Symmetric	0.637	Leptokurtic
U3	End	Unlimited	Yes, P = 0.3877	-0.886	Moderately left	-1.045	Platykurtic

Table 2. Colonies L1-L3 received limited resources while colonies U1-U3 received unlimited resources. This table shows whether size distributions of newly emerged workers were normally distributed for each colony for each growth period (beginning, middle, and end). The values and descriptions for sample skew and sample excess kurtosis values over colony growth are also shown.

Figure 2.

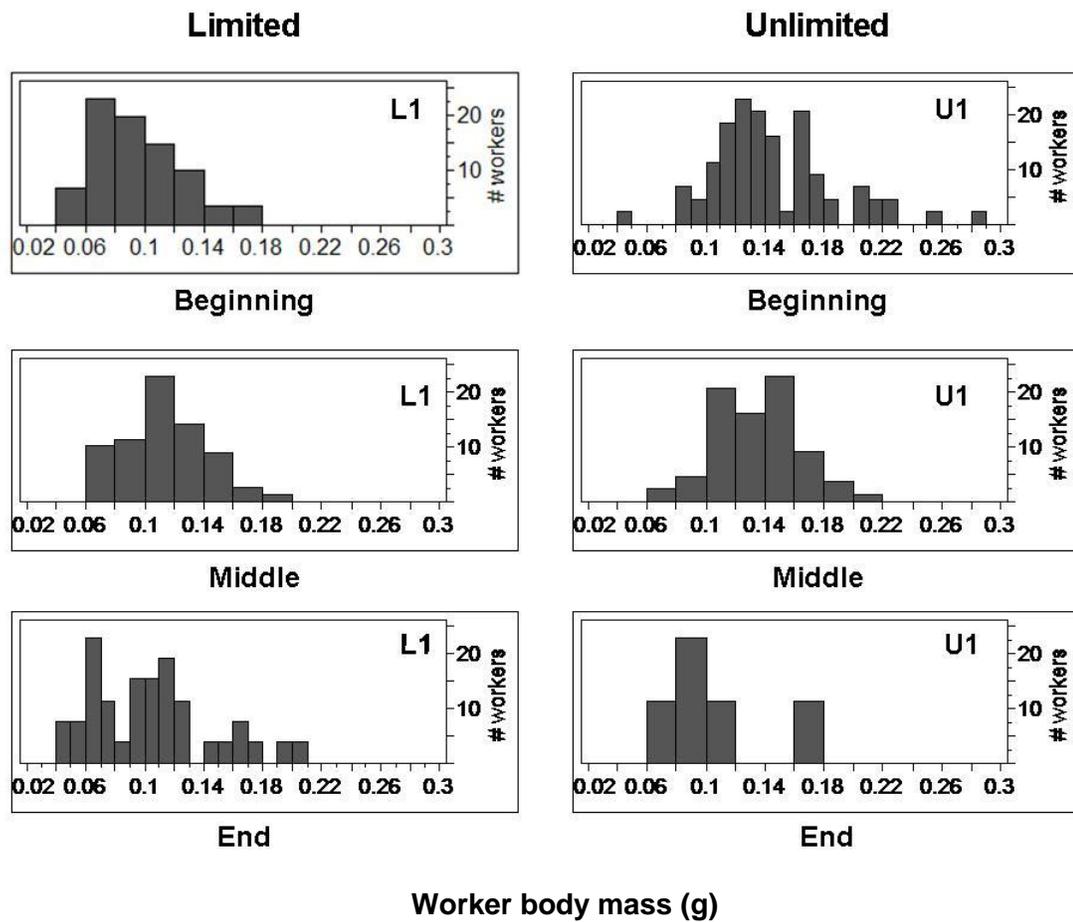


Figure 2. Sample frequency distributions for body masses of newly emerged workers in the beginning, middle, and end of colony growth for colonies which received limited resources (Colony L1) and unlimited resources (Colony U1). Letters within each panel indicate colony. These two colonies were randomly chosen to show how size distribution of worker mass looks like throughout colony growth.

Figure 3.



Figure 3. The average worker mass \pm SE for each resource availability treatment across colony growth. Black bars with upper case letters present colonies in limited resources ($N = 3$) and grey bars with lower case letters present colonies in unlimited resources ($N=3$). Different letters indicate significant differences in average worker mass ($P < 0.05$).

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APPENDIX C

WHAT IS A BEE'S WORTH? THE PRODUCTION COSTS AND WORK OUTPUT
OF DIFFERENT SIZED BUMBLE BEE (*BOMBUS IMPATIENS*) WORKERS

**What is a bee's worth? The production costs and work outputs of different sized
bumble bee (*B. impatiens*) workers**

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Summary

Body size is an important life history trait because of its often high correlation to fitness. Variation in body size can be very large in some social insects. For example, worker body mass can vary up to 500 fold in the Asian marauder ant (*Pheidologeton diversus*) or up to 10 fold in bumble bees (*Bombus* spp.) whereas worker mass can vary no more than two fold in other bees. While there is strong evidence that the worker morphology and size variation is beneficial for task specialization in some social insects, such as leaf-cutter ants (*Atta* spp.), it is not fully clear why such large worker size variation exists in bumble bees (*Bombus* spp). To gain a better understanding of why worker size variation exists, we investigated the energetic costs and work benefits of different sized bumble bee workers. Worker production can be viewed as a colony-level investment in which larger workers cost more energy to produce than smaller workers. Social insect workers do not reproduce themselves; they gain inclusive fitness by expending their own energy working and contributing towards colony-level growth and rearing reproductively-capable siblings. With this in mind, do workers ‘pay-off’ their production costs through their lifetime work efforts? We found that larger bumble bee (*B. impatiens*) workers have higher energy content, suggesting that they have higher production costs. There were no effects of body size on lifetime work efforts, which we measured both as the proportion of a worker’s lifetime spent actively performing a task and as the energy spent working throughout its entire lifetime. Body size also did not affect work intensity, the amount of energy spent working/day. However, larger foragers brought in more pollen and nectar and hence, more energy resources into the colony. This suggests that smaller workers are reared because they are cheap to produce and work just as hard per day and as much in a

lifetime as larger workers, but larger workers are reared because they bring in more energy resources into the colony. In addition, workers from colonies with more food had higher lifetime work efforts and work intensities, probably because colonies are able to rear more brood, which leads to more work. Lastly, there was a significant negative correlation between work intensity and worker lifespan.

Introduction

Body size is an important life history trait and it is generally an indicator of fitness. This is true for many animals, including insects. For instance, larger individuals provision or collect more food (Alcock 1979; Johnson 1990; Goulson *et al.* 2002), forage at higher rates (Spaethe & Weidenmüller 2002), have higher fecundity (Molumby 1997), have more success in obtaining mating territories (Alcock 1990), and have longer lifespans (Speakman 2005b). However, small body size may have fitness advantages as well (Blanckenhorn 2000), and this could lead to body size variation within populations. For instance, small size in Dipteran males is related to higher success in mating, probably due to better agility (McLachlan & Allen 1987; Steele & Partridge 1988). In addition, less food provisioning and energy would be involved in producing smaller individuals, since more food provisioning and energy is involved in producing larger individuals (Bosch & Vicens 2002), which would be an advantage to the provisioning parent. Variation in body size among individuals thus arises because of the relative costs and benefits that are associated with a particular size and the environmental context that influences size.

Variation in body size can be very large among related individuals in some social insect colonies. For example, body mass can vary up to 500 fold among workers in the

Asian marauder ant, *Pheidologeton diversus* (Hölldobler & Wilson 1990) or up to 10 fold in bumble bees (*Bombus* spp.) (Alford 1975) whereas worker mass varies no more than two times in other bees (Waddington, Herbst & Roubik 1986; Ramalho, Imperatriz-Fonseca & Giannini 1998; Roulston & Cane 2000). While there is strong evidence that worker morphology and size variation is beneficial for task specialization in some social insects, such as leaf-cutter ants (*Atta* spp.), it is not fully clear why such large worker size variation exists in bumble bees (*Bombus* spp). Bumble bees are social insects, and their colonies are primarily comprised of non-reproducing sister workers who contribute their labor towards colony-level reproduction, and ultimately, their own inclusive fitness (Hamilton 1964). Workers perform tasks such as nest defense, foraging, brood care, nest thermoregulation, hygienic tasks, etc. All of these tasks effectively contribute towards a colony's ability to acquire energy resources and translating the acquired resources into brood which eventually include the reproductives (virgin queens and males). In bumble bees, division of labor is associated with body size, where larger bumble bee workers are usually foragers and smaller workers perform within-nest tasks (Goulson *et al.* 2002; Jandt & Dornhaus 2009a), although workers can switch among tasks (Jandt, Huang & Dornhaus 2009a).

Quantity of nutrition (Plowright & Jay 1977; Sutcliffe & Plowright 1988b; Couvillon & Dornhaus 2009a) and spatial location (Couvillon & Dornhaus 2009a) during larval development influence bumble bee worker size. The more food a larva receives, the larger it would be as an adult (Plowright & Jay 1977; Sutcliffe & Plowright 1988b). In addition, larvae in the center of the nest are fed more frequently and are thus larger than those along the nest periphery (Couvillon & Dornhaus 2009a). Differential worker

size could thus present different colony-level investments towards colony-level growth and reproduction. To better understand why colonies produce workers of various sizes, we investigated the general relationship among worker body size, production costs, lifespan, and work output. By viewing the colony as an entity that invests in workers and quantifying how these investments are ‘paid-off’, we could gain a better understanding of why bumble bee colonies have such large worker size variation. Workers can ‘pay-off’ their production costs and contribute to colony growth and reproduction by the amount of work they perform within their adult lifespans. Larger foragers should also collect more food resources for colony use, as it has been shown that larger foragers (*B. terrestris*) carry greater nectar loads (Goulson *et al.* 2002) and forager for nectar at higher rates (Spaethe & Weidenmüller 2002). It would be expected that larger workers cost more energy to produce and because of this, they should do more work within their lifetimes and have higher work intensity (i.e. do more work/day throughout their lives).

We ask the following questions: 1) what are the production costs of different sized workers, 2) considering these costs, does body size influence the amount of work performed within workers’ lifetimes, and their work intensity 3) do larger foragers bring in more resources (pollen and nectar loads) into the colony, and 4) because the amount of food affects worker body size (Plowright & Jay 1977; Sutcliffe & Plowright 1988b) and colony population size and growth rate (unpublished data), does resource availability influence worker production costs, lifetime work effort, and work intensity?

Methods

Although costs of producing workers should include energy spent by workers in rearing brood (e.g. collecting food, feeding, thermoregulating, etc.), and the amount of

food that is consumed by individuals, body size itself is a good estimate of production costs in bees (Bosch & Vicens 2002). Here, we quantified the energy content of a sample of various sized workers using bomb calorimetry. To estimate how much food (i.e. energy resources) foragers could bring into the colony, we measured the foraging loads of various sized workers. Lifespan is important to consider, as it is the timeframe in which workers potentially contribute work towards the colony. In addition to lifespan, we estimated lifetime work effort behaviorally and energetically by calculating estimates for the proportion of workers' adult lifespans spent being active and for energy expended while working throughout their lives, respectively. Thermal imaging was used to estimate energetic costs of performing various tasks and this data was used to generate lifetime energy budgets. Although directly costly to the worker, we considered the energy spent working as contribution towards colony growth and reproduction and ultimately, a worker's inclusive fitness.

Calorimetry – estimating production costs

Two colonies were purchased from Koppert Biological Systems (Romulus, MI, USA) in October 2012. One received unlimited access to food while the other received limited access to food (more details on resource availability treatment set up is described below in the 'behavioral experiments' section). Each colony was connected to a foraging arena (3 m long X .61 m high X .61 m wide). Daylight lamps were connected to the end of each foraging arena and set to a day:night cycle of 12:12 hours. Colonies were checked daily for newly emerged workers; all newly emerged workers were collected and their whole body wet masses were measured using a balance (Mettler Toledo XS3DU). They were then placed in sealed plastic containers and frozen. The bees were oven dried for three

days and their dry masses were measured. The energy content (calories) of the dry bees were measured using bomb calorimetry (Parr calorimeter (6200 with water handling system (6510), using the 1108 bomb, spiked with 0.5 ml mineral oil (Ace Hardware). Calories were converted to joules by multiplying by 4.184.

Foraging load experiments

Two *Bombus impatiens* colonies were purchased from Koppert Biological Systems (Romulus, MI, USA) in August, 2012. The colonies were set up under laboratory conditions at the University of Arizona in Tucson, Arizona. Workers were marked with color number tags for identification. Daylight lamps were connected to the end of each foraging arena and set to a day:night cycle of 12:12 hours. Both colonies were fed daily and received unlimited access to food. Each colony was connected to a foraging arena through a 3 m long X .61 m high X .61 m wide flight tunnel through a clear y-shaped tube. One pathway of the tube connected the nest directly to the foraging arena while the other led to an opening to where a researcher collected returning foragers. Colonies were observed from 9 AM to 3 PM. Any foragers returning to the nest were blocked from entering the nest and from re-entering the foraging arena by two separate cardboard doors that fit into slits through the y-tubing in front of the entrances to the nest and foraging arena. This forced foragers to enter through the opening that led to the other pathway of the y-tubing and into the researcher's collection tube. Foragers were then chilled on ice. Once a forager stopped moving, any pollen was carefully removed from its corbiculae with forceps and weighed. To weigh a nectar load, a forager's proboscis was extended into an Eppendorf tube and its abdomen was squeezed until all, if any, crop contents were expelled; the contents were then weighed. The worker was then returned to the colony

and not collected again in that same day. When possible, multiple measures were collected from the same workers on different days; in such cases, the maximum values for pollen or nectar loads were used for analyses.

Behavioral experiments- quantifying lifetime work efforts and adult lifespan

Six colonies of *Bombus impatiens* were purchased from Koppert Biological Systems (Romulus, MI, USA) in August 2011, and set up in a greenhouse located at the University of Arizona in Tucson, Arizona. Upon arrival, all six colonies were set up in clear plastic nest boxes (10 cm tall x 18 cm wide x 29 cm long), covered with a Plexiglass top, and lined with pine kitty litter. Colonies were set up to simulate conditions that were near the founding phase such that each colony had 1 queen, about 12 workers, and about 10 food cells. No brood were included in the colonies at set-up to ensure that any individuals that emerge from these colonies were reared under the conditions of the food treatment. For the first 5 days, colonies were given nectar solution (50% volume of Bee Happy, 50% volume of water) and pollen (Koppert Biological Systems, Romulus, MI, USA) *ad libitum* in their colonies to allow for recovery from the shipping process and successful acclimation to their new colony setup in the greenhouse. Because eggs take about 5 days to hatch (Goulson 2003b), there should not be any larvae exposed to food treatments in these first 5 days of acclimation.

After the fifth day, colonies were randomly assigned to one of two resource availability treatments: ‘unlimited’ and ‘limited’, three colonies per food treatment. Colonies in the limited resource treatment were labeled as L1, L2 and L3 for identification, while colonies in the unlimited resource treatment were labeled as U1, U2, and U3. In the unlimited treatment, colonies were given access to pollen and nectar

solution 24 hours a day, whereas colonies in the limited food treatment were given access to pollen and nectar solution for 5 hours per day. The 24 hours of resource availability in the unlimited resource treatment ensured that every forager had access to food during foraging times, which occurred during daylight hours. Daylight ranged from about 10-12 hours throughout the whole experiment. Thus, foraging time for workers in the limited resource availability treatment was about 42%-50% of the foraging time that workers in the unlimited resource availability treatment had. Each individual colony had access to their food in a foraging arena, connected to the colony entrances via a 0.61 m wide x 0.91 m high x 8.2 m long flight tunnel. These large foraging arenas required workers to fly during foraging, simulating natural foraging processes and allowed us to keep workers of different food treatments separated from each other. Pollen and nectar solutions were placed on a platform (~ 0.46 m tall) at the opposite end of the flight tunnel from the colony; two blue and yellow artificial flowers were placed near the food platform in each foraging arena as visual cues to encourage successful detection of food by the bees. Strips of yellow paper were fixed to the vertical sides of the flight tunnel to provide visual cues to aid in flight during foraging. Access to food was denied in the limited food treatment simply by removing food from the foraging arena. The masses of pollen and nectar solution that colonies consumed were measured at least every other day. The experiment ran for 100 days, counting from the first day of colony set up.

Colonies were checked daily for newly emerged workers; these newly emerged workers were marked with color-number tags using super glue. This identification tag allowed us to identify focal workers and keep track of their lifespans. We used the scanning method to generate life history work profiles for all the workers in our study.

Behavioral scans consisted of observational periods during which a researcher scanned the colonies systematically from the left to right sides, beginning from the top. Upon identifying a focal bee during the scan, the task she was performing at the time was recorded for that observation period. The researcher also scanned the foraging arena for foragers who were not in the nest. Behavioral scans were conducted for all colonies four times a week (Monday, Tuesday, Thursday, and Friday), with five observational periods per day conducted between 8 AM- 5 PM. A total of 14 different tasks were recorded in our study (Table 1). Only workers with at least 30 observation periods were used for analyses (sample size of workers per colony: Colony L1 = 38, Colony L2 = 36, Colony L3 = 46, Colony U1 = 56, Colony U2 = 67, Colony U3 = 33).

Colonies were checked daily for newly emerged workers and the body mass of each newly emerged worker was measured using a digital scale to the nearest 0.001 g. Measuring the mass of newly emerged bees controlled for age effects on body mass. Prior to measuring body mass of all the newly emerged bees, individuals were chilled in a refrigerator for about 10-15 minutes; this was done so that individuals were not moving on the scale during mass measurements. To ensure that honey crops were empty, individuals' abdomens were squeezed prior to measuring body mass. Large workers were easily distinguishable from virgin queens because there is little overlap in body size between workers and queens in pollen-storing bees, which include *B. impatiens* (Goulson 2003b). Nests were checked daily for dead workers, and workers' lifespans were calculated as the number of days they were alive as adults, beginning from the day of emergence to the day of death.

Thermal Experiments-estimating energetic costs of working

Two *B. impatiens* colonies were used for this experiment (Koppert Biological Systems, Romulus, MI) and kept under laboratory conditions at 28 °C. Two large windows allowed natural daylight into the laboratory, but to ensure consistent lighting, daylight bulbs were set on timers at the end of each foraging arena so that day:night ratio would be 12:12. Each colony was connected to a flight tunnel (0.61m wide x 0.61m high, x 3.05m long) that encouraged foragers to fly over a distance to collect food. Temperature data was recorded using an infrared camera (FLIR T300). Workers were observed in their nests through an infrared window that was 101.6 mm in diameter (IRISS, model VPT-100) that was placed over a 76 mm diameter opening in the Plexiglass top of the colony, allowing for thermal images of working bees to be captured. The highest temperatures in active workers were observed to emanate from thoraces; thus, maximal temperatures generated from the thoraces during certain tasks were used as estimates of the energy associated with performing that task. It was not possible to capture infrared images of bees in flight, so data for foraging was estimated using a type T 0.02 mm diameter thermocouple (Hyop-33-1-T-G-60-SMPW-M, Omega) connected to an Omega HH23A thermocouple reader that was inserted from the dorsum into the thoracic muscle of workers (N = 17). The thermal couple was secure enough to support a worker's body during flight. To encourage flight, a worker was simply lifted into the air. The flight temperatures typically reached maximum values after three minutes; these maximum temperatures were used to estimate flight cost, and hence, foraging costs.

One caveat of using thermal imaging is that thermal imaging does not capture the energy that is dissipated during task performance, especially for fanning and foraging. Measuring rates of oxygen consumption or carbon dioxide production through

respirometry for bees performing the various tasks would be a more accurate method of measuring costs of task performance, but this would have been impractical to do for all 14 tasks that were observed in this study (Table 1). Thermal imaging provided us with a practical way to estimate the energetic costs related to performing the 14 tasks that were observed in this study.

Lifetime activity budgets and energy budgets

Data from the behavioral observation scans were used to generate lifetime activity budgets for all workers. The unit we use here for our energy budgets is proportion. A worker's proportion of its lifetime that was spent performing a certain task (Table 1) was calculated as: total number of observation periods spent performing task 'X'/total number of observation periods in which the worker was present. Only workers with at least 30 observation periods were used for analyses (sample size of workers per colony: Colony L1 = 38, Colony L2 = 36, Colony L3 = 46, Colony U1 = 56, Colony U2 = 67, Colony U3 = 33).

Energy budgets were generated from the activity budgets and the amount of energy associated with performing a task. Based on the number of times a worker was observed performing each of the 14 tasks (Table 1), these energy budgets calculated the amount of energy that a worker spent working in its lifetime. The amount of energy associated with performing a particular task was calculated from thermal data obtained from the thermal experiments (described above). The thermal data were converted to energy units, joules, by using the following equation that calculates heat energy: $Q = T_m c \Delta T$, where Q = heat energy, T_m = thoracic mass, c = specific heat, and ΔT = change in temperature ($T_{\text{activity}} - T_{\text{rest}}$). The specific heat for insect tissue is 0.8 cal/g·°C (Heinrich 1975;

May 1976). Because workers in this thermal experiment were left intact and returned to the nest after each total body mass measurement, their thoracic masses were estimated using a linear prediction equation. This linear prediction equation was calculated based on fresh weight data for body mass and thoracic mass of bees from a separate experiment. The prediction equations was: thorax width = $0.391 + 0.450 \cdot \text{whole body mass}$ (Simple linear regression: $t_{33} = 35.12$, $P < 0.0001$, $R^2 = .97$). The estimated thoracic mass for workers in this study was then used in equation, $Q = T_m c \Delta T$, to calculate energy (or heat) production for a particular task. In addition, because it was not feasible to measure the resting temperature (T_{rest}) of each worker before recording the temperature during a particular task (T_{activity}), an average resting temperature was used for all workers. Assuming that bumble bee workers' body temperatures at rest (T_{rest}) are the same as the nest ambient temperature, multiple temperature data of the nest ambient temperature were recorded using a type K thermocouple connected to an Omega HH23A reader. For each colony, two measurements were recorded in each corner of the nest and two from the center of the nest. The average ambient nest temperature for colony A was $28.6 \pm .065$ °C and $29.4 \pm .20$ °C for colony B (N = 10 temperature recordings for each colony); these two values were used as resting temperatures for bees from each colony. Temperature data while performing certain tasks (Table 1) from both colonies were pooled and median temperature values were calculated for tasks with sample sizes greater than four temperature values. Median values were not calculated for chewing, digging, and undertaking tasks because sample sizes were less than four temperature values; these tasks were difficult to capture as infrared images either because the tasks were performed too briefly or were rarely performed. Thus, the final energy budgets calculated for workers are conservative calculations.

Statistics

All means are reported as mean \pm SE unless otherwise stated. Only bees with at least 30 behavioral observation periods were used in analyses. Analyses were conducted using JMP (JMP®). Data were normally distributed (Shapiro-Wilk test, $P \geq 0.5$) unless otherwise stated.

Body mass of workers ranged from 0.046 g to 0.309 g. It was not possible to collect sufficient sample for each of the 14 tasks for the full range of bee sizes. Therefore, quantiles of worker body mass were used to categorize body size into four size groups: small (0.046-0.0101 g), medium (0.102-0.124g), large (0.125-0.154), and extra large (0.155-0.309 g). Lifetime work effort was estimated in two ways: 1) as the proportion of the worker's lifetime spent being active and 2) as the amount of energy spent working throughout its lifetime. The proportion of a worker's lifetime spent working was calculated as the number of observational periods performing any task other than being inactive divided by the total number of observational periods in which the worker was identified. Proportion of observation times spent being active was arcsine transformed to normalize the data. The transformed data was used in a two-way ANOVA to test effects of body size and colony on workers' proportion of time spent performing a task other than being inactive. A mixed model ANOVA with body size and resource availability treatment as fixed variables and colony as a blocking variable resulted in the loss of degrees of freedom. Thus, a two-way ANOVA was used instead and followed up with the LSMeans contrast test, in which colonies L1, L2, and L3 (limited resource availability treatment) were contrasted against colonies U1, U2, and U3 (unlimited resource availability).

Residuals for the amount of energy spent throughout workers' lifetimes were not normally distributed for all levels of body size (Shapiro-Wilk test, $P \leq .05$). Data were rank transformed and a two-way ANOVA on ranks was performed to test whether energy spent throughout a worker's lifetime was affected by body size and colony. This test was also followed by an LSMeans contrast test for the colony variable to determine if resource availability treatment influenced lifetime work effort.

To estimate how 'hard' workers worked per day throughout their lives, 'work intensity' was calculated as lifetime work effort, measured as energy (joules), divided by adult lifespan (days). Residuals for work intensity (joules/day) were not normally distributed for all levels of colony and body size (Shapiro-Wilk test, $P \leq .05$). Thus, the work intensity (joules/day) data were rank transformed and a two-way ANOVA on ranks was performed to test whether colony and body size influenced work intensity. This was followed by an LSMeans contrast, similarly as to the analyses above.

Lifespan data were not normally distributed (Shapiro-Wilk test, $P \leq 0.05$) and were rank transformed. To determine if lifespan was associated with work intensity and resource availability, a multiple regression test was performed on ranks, with colony as a blocking variable.

Results

Association between body mass and energy

The energy content of workers was significantly positively correlated with their dry body mass (Fig. 1). Resource availability did not have a significant effect on the whole energy content of workers (Multiple regression: dry mass, $F_{1, 31} = 343.12$, $P < 0.0001$; resource availability, $F_{1, 31} = 0.53$, $P = 0.47$, $R^2 = .92$). The multiple regression equation for workers from the limited treatment is: energetic content = $4.94 + 5198.91 * \text{whole body dry mass} + 1.36$. The multiple regression equation for bees from the unlimited treatment is: energetic content = $4.94 + 5198.91 * \text{whole body dry mass}$.

Forager body mass and foraging load

There was marginally significant correlation between body mass and maximum pollen load (Fig. 2A), and there was no significant colony effect on pollen load (Multiple linear regression: body mass, $F_{1, 23} = 4.28$, $P = 0.05$, $b = 0.089$; colony, $F_{1, 23} = 0.12$, $P = 0.74$, $R^2 = 0.17$; $N = 26$ bees). There was a significantly positive correlation between body mass and nectar load (Fig. 2B), but there was no significant colony effect (Multiple linear regression: body mass, $F_{1, 61} = 57.07$, $P < 0.0001$, $b = 0.40$; colony, $F_{1, 61} = 1.55$, $P = 0.22$, $R^2 = 0.50$, $N = 64$ bees). The regression equation for pollen load mass is: pollen load mass = $1.04 + 0.089 \cdot \text{body mass}$. The regression equation for nectar load mass: nectar load = $-14.19 + 0.40 \cdot \text{body mass}$.

Lifetime work effort

A. Proportion of lifetime spent being active

Colony had significant effects on the proportion of observation periods in which workers were actively performing a task such that colonies in the unlimited resource availability treatment ($N = 3$ colonies) were more actively working than those in colonies that received limited resources ($N = 3$ colonies) (LSMeans contrast test: $F_{1, 252} = 12.96$, $P = 0.00038$). There were no significant body size or colony x body size effects on the proportion of observation periods in which workers were active (two-way ANOVA: body size, $F_{3, 252} = 1.53$, $P = 0.21$; colony, $F_{5, 252} = 4.45$, $P = 0.00070$; colony x body size, $F_{15, 252} = 1.03$, $P = 0.43$).

B. Energy spent throughout workers' lifetimes

Body size was not associated with lifetime work effort (Fig. 3), measured as the total energy spent working throughout all the observation periods in a worker's lifetime. However, resource availability significantly affected lifetime work effort such that lifetime work effort was higher in the unlimited resource treatment (Fig. 4; LSMeans contrast test: $F_{1, 268} = 11.64$, $P = 0.00075$). There was no significant colony x body size effect on lifetime work effort (two-way ANOVA on ranks: body size, $F_{3, 251} = 1.64$, $P =$

0.18; colony, $F_{5, 251} = 3.90$, $P = 0.0020$; colony x body size, $F_{15, 251} = 1.15$, $P = 0.31$, $R^2 = 0.20$).

Work intensity

Work intensity for each worker was calculated as the total energy spent throughout its entire adult life divided by its adult lifespan. There were significant colony effects on work intensity such that workers in colonies with unlimited resources (N = 3 colonies) spent more energy working per day than those in colonies with limited resources (N = 3 colonies) (LSMeans contrast test: $F_{1, 252} = 15.33$, $P = 0.00012$). There were no significant body size or colony x body size effects on work intensity (two-way ANOVA on ranks: body size, $F_{3, 252} = 0.52$, $P = 0.67$; colony, $F_{5, 252} = 8.63$, $P < 0.0001$; colony x body size, $F_{15, 252} = 0.77$, $P = 0.71$).

Lifespan

Lifespan was significantly negatively correlated with work intensity (Fig. 5); resource availability did not affect lifespan (multiple regression on ranks, blocked by colony: work intensity, $F_{1, 240.9} = 18.69$, $P < 0.0001$; resource availability, $F_{1, 3.90} = 1.84$, $P = 0.25$, N = 276 bees).

Discussion

As would be expected, larger workers had higher energy content than smaller workers: it costs the colony more to produce a larger worker than a smaller worker. For the colony to recoup this larger investment, it was found that larger workers should have higher lifetime work efforts than smaller workers. However, we found that body size did not affect lifetime work effort, whether it was measured as the proportion of a worker's lifetime being active or the energy spent working throughout its lifetime. Body size also

did not affect work intensity, or the amount of energy spent working per day. We found that of the bees who foraged, the larger foragers brought back larger foraging loads, and hence, more energy resources into the colony. Thus, although larger workers cost more energy to produce but have similar lifetime work efforts and work intensities as smaller and cheaper workers, the colony recoups this investment through the larger bees bringing in more energy resources into the colony.

Our results showed that lifetime work effort, whether it be measured as proportion of lifetime spent working or energy spent doing work throughout a worker's lifetime, was higher in colonies that received unlimited resources. In addition, workers from colonies that received unlimited resources had higher work intensity (i.e. energy spent working/day) than those that received limited resources. Bumble bee colonies that receive more food have larger population sizes and reach reproduction at a faster rate than colonies that receive less food (Cao & Davidowitz, unpublished data). It is possible that because there was more food available to these colonies, workers were able to forage more and use the collected resources to rear more brood, and hence, do more work within the nests. It has been suggested that increase in work load may reduce lifespan in social insect workers (Schmid-Hempel & Wolf 1988b; Wolf & Schmid-Hempel 1989). Our results support this prediction: workers with higher work intensity (i.e. energy spent working/day) had reduced lifespans.

In bumble bees (Goulson *et al.* 2002; Goulson 2003b; Jandt & Dornhaus 2009a), some ants such as the leafcutter *Atta* (Wilson 1980), and termites (Traniello & Rosengaus 1997), body size is the main predictor of task performance. In fact, some ants and termites have different and morphologically distinct worker castes, individuals who are

presumably physically/physiologically adaptive to the tasks that they perform. For instance, in the leaf-cutting ants, *Atta*, the smallest workers work in fungal gardens, medium-sized workers forage for food, and the largest worker with enlarged mandibular muscles defend the nest (Wilson 1980). In such cases, worker body size variation is typically multi-modal and/or skewed (Davidson 1978; Wetterer 1999). In bumble bees, workers vary up to 10-fold in body mass (Alford 1975), but worker size variation within nests is usually normally distributed rather than discontinuous or skewed (Couvillon *et al.* 2010). This variation in worker mass is much larger than that of social insects, which are typically less than a two-fold variation in body mass (Waddington, Herbst & Roubik 1986; Ramalho, Imperatriz-Fonseca & Giannini 1998; Roulston & Cane 2000; Goulson *et al.* 2002). Unlike some ants and termites, it is still unclear why there is large variation among workers and whether this variation is beneficial for division of labor *per se*. By assessing the energetic costs and behavioral benefits associated with different sized workers, we may enhance our understanding as to why such a large variation in worker body size occurs within bumble bee colonies.

Based on our results, smaller workers are energetically cheaper to produce and do just as much work as larger workers. However, they do not collect as much pollen and nectar as larger workers. Thus, both smaller and larger workers are produced, leading to the large worker size variation observed in colonies. Furthermore, it is possible that the normal distribution of worker size, with most workers being of average body size, reflects a balance among production costs, lifetime work effort, and foraging efforts. Our results provide insight as to how energetics may at least partially influence worker size distribution within bumble bee colonies.

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Figures and Captions

Table 1.

Task	Description	Median energy value associated with each task (joules/observation of task)
Chewing nest lining	Worker was chewing the nest lining, with her head moving side to side.	N/A
Defense	If the worker walked really fast around the nest for minutes after a colony was accidentally disturbed or if a worker was flying around the nest but did not leave to forage.	0.944
Digging	Worker had her head down towards the nest lining and she was digging the nest lining from underneath her body.	N/A
Drinking honey	Worker had her proboscis extended and her abdomen was not contracting as foragers do when excreting nectar into honey pots. Also did not feed larvae within 10 seconds after drinking honey.	1.654
Fanning	Worker fanned her wings silently (not buzzing).	1.310
Feeding larvae	If worker was chewing a hole through a larval cell or had her proboscis extended into a larval cell. Also if a worker was drinking from a honey pot but 10 seconds later regurgitates into a larval cell.	1.417
Foraging	A worker that was seen leaving the nest, returning to the nest, or seen in the foraging	2.422 (estimated using data from flying bees)

	arena.	
Grooming	Worker who rubbed her legs together or if her forelegs brushed over her head and thorax.	1.697
Inactive	Worker was motionless inside or outside of the nest for >30 seconds. She was not actively looking for or performing work.	0
Incubation	A worker was wrapped around a cell and her abdomen was moving in a pump-like motion.	2.073
Inspecting	Worker was walking on top of the brood comb, antennating various parts of the waxy nest structure for at least 10 seconds.	1.609
Probing honey pot	Worker dipped her head into a full honey pot.	1.702
Undertaking	Worker carried dead workers, pieces of dead workers, or dead larvae away from the nest	N/A
Wax handling	Worker used her mandibles to chew on pieces of wax on the comb to reshape the pieces.	1.697

Figure 1.

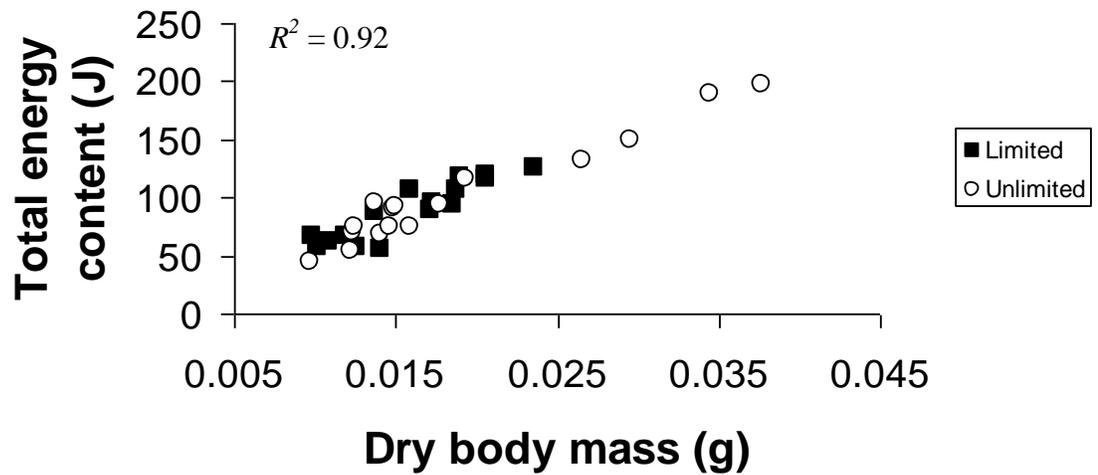


Figure 1. Whole body dry mass (g) and energetic content (joules) of bumble bee workers. Square data points are bees from the colony that received limited resource availability and circle data points are bees from the colony that received unlimited resources. Dry mass was significantly positively correlated with energetic content ($R^2 = 0.92$, $P < 0.0001$). Multiple regression equation energetic content = $4.94 + 5198.91 \times \text{whole body dry mass} + 1.36$. Multiple regression equation for unlimited treatment: energetic content = $4.94 + 5198.91 \times \text{whole body dry mass}$.

Figure 2.

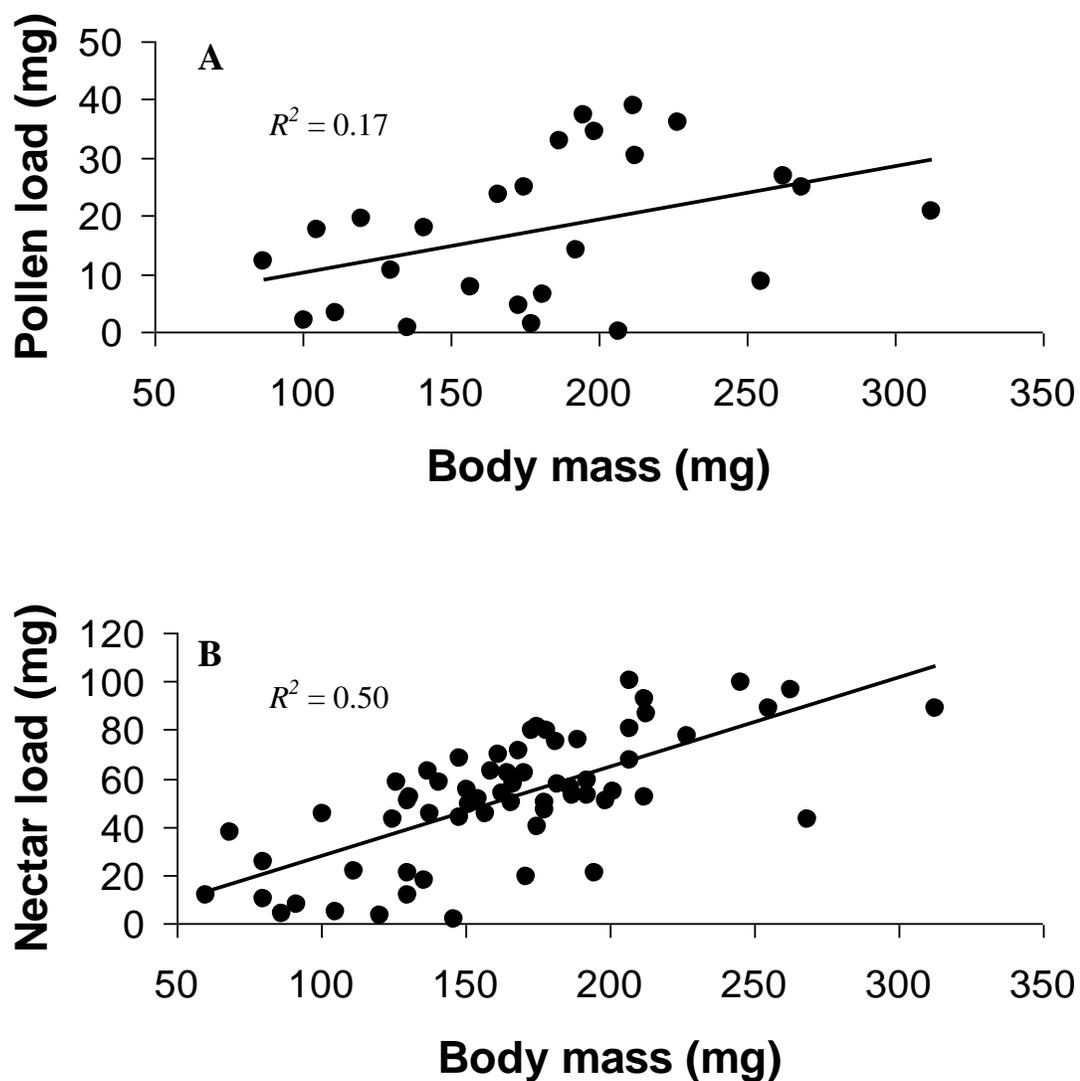


Figure 2. Worker body mass and pollen load were marginally positively correlated (A, $R^2 = 0.17$, $P = 0.05$). Body mass and nectar load were significantly positively correlated (B, $R^2 = 0.50$, $P < 0.0001$). Each dot represents a bumble bee worker. Regression equation for pollen load mass (A): pollen load mass = $1.04 + 0.089 \times \text{body mass}$. Regression equation for nectar load mass (B): nectar load = $-14.19 + 0.40 \times \text{body mass}$.

Figure 3.

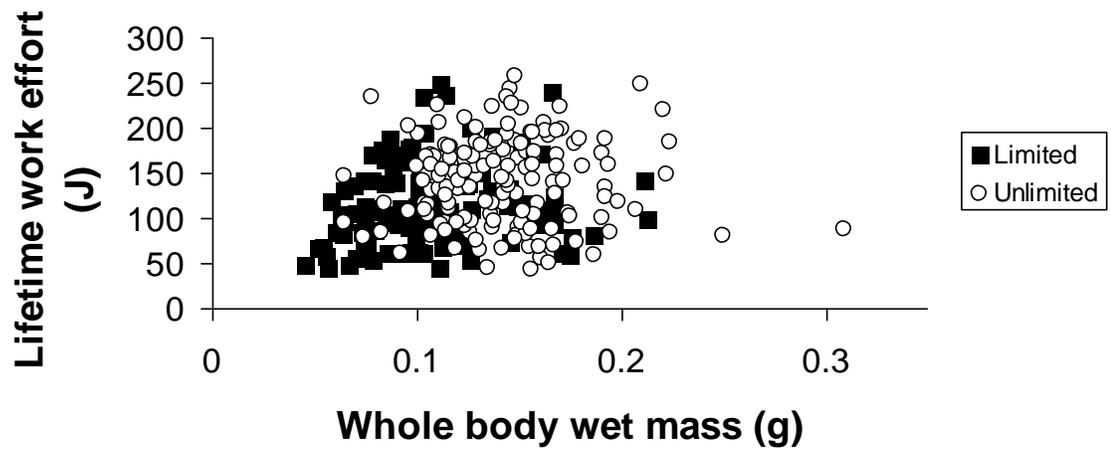


Figure 3. Body mass and lifetime work effort, estimated as energy spent throughout a worker's lifetime (joules). Square data points represent workers in the limited resource availability treatment while circle data points represent workers in the unlimited resource treatment.

Figure 4.

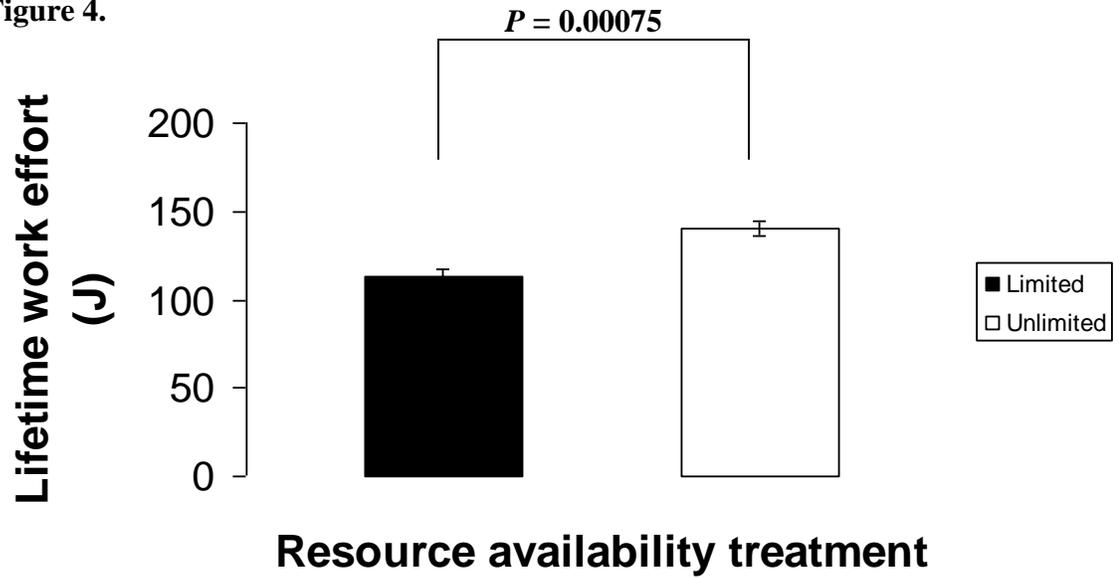


Figure 4. Lifetime work effort, as energy spent throughout workers' lifetimes (joules) was significantly higher in colonies with unlimited ($N = 3$ colonies) resources than in those with limited resources ($N = 3$ colonies).

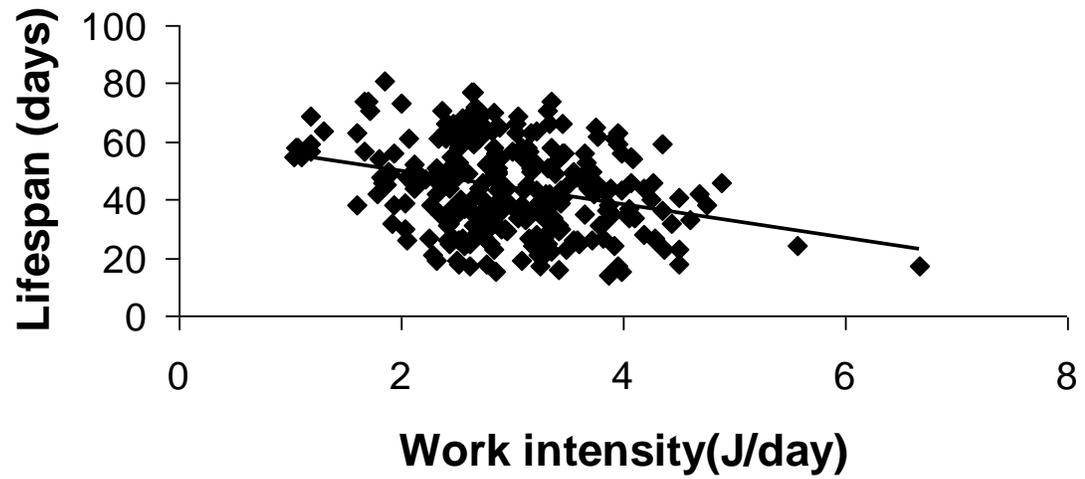
Figure 5.

Figure 5. Adult lifespan (days) was significantly negatively correlated with work intensity ($R^2 = 0.12$, $P < 0.0001$), calculated as the total energy spent throughout a worker's lifetime/adult lifespan. Each data point presents a worker bee ($N = 276$).

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APPENDIX D

METABOLIC RATE DECREASES WITH AGE AND AFFECTS THE LIFESPAN OF
BUMBLE BEES, *BOMBUS IMPATIENS*

Metabolic rate decreases with age and affects the lifespan of bumble bees, *Bombus impatiens*

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Summary

The rate of living theory posits that metabolic rate is negatively correlated with lifespan, with the notion that higher metabolic rates accelerate the rate of aging. However, correlations between resting metabolic rate and lifespan within species are inconsistent. In addition, many studies involve single measurements of resting metabolic rates and assume that they remain constant throughout ontogeny. In this study, we use the bumble bee *Bombus impatiens* to test whether resting mass-specific metabolic rate affects potential lifespan, i.e. lifespan where mortality from predation or extreme weather changes is eliminated, and whether resting mass-specific metabolic rate changes with age. We measured resting mass-specific metabolic rates of workers on the first day post-emergence and tracked their potential lifespans. We also made repeated metabolic measurements throughout workers' lives. Our results show that (1) resting mass-specific metabolic rate is negatively correlated with potential lifespan, and (2) resting mass-specific metabolic rate decreases with age. These results imply that workers with higher resting mass-specific metabolic rates, i.e. higher maintenance costs, have shorter potential lifespans. In addition, workers' maintenance costs generally decrease as they become older and they show metabolic aging.

Introduction

Why do some individuals die sooner than others? One of the most debated theories for what affects lifespan and aging is the 'rate of living' theory, which was properly formulated by Pearl (1928). This theory posits that lifespan and aging are affected by the rate of energy metabolism (Pearl 1928; Beckman & Ames 1998; Speakman 2005; Melvin, Van Voorhies & Ballard 2007). Aging (or senescence) refers to a decline in physiological function with time, which is coupled with an increase in mortality rate and a decrease in reproduction (Rose 1991). Energy metabolism is used as a measure for living costs (Schmidt-Nielson 1991). Although there has been some support for the hypothesis that metabolism is inversely related to lifespan (Pearl 1928; Sohal 1986; Calabi & Porter 1989; Beckman & Ames 1998; Speakman 2005), there has also been support for the opposite relationship in which the relationship between metabolism and lifespan are positively related (Møller 2008) as well as no relationship between

metabolism and lifespan, such as in some dipterans (Hulbert *et al.* 2004; Hulbert, Usher & Wallman 2004; Melvin, Van Voorhies & Ballard 2007). In this study, we test the hypothesis that there is an inverse relationship between resting metabolic rate and lifespan. Resting metabolic rate is a standardized measurement that indicates an individual's maintenance cost, which is a central life history trait (Burton *et al.* 2011). Maintenance costs can make up to 50% of an individual's total daily energy expenditure (Congdon, Dunham & Tinkle 1982). We used a social insect whose unique biology allowed us to naturally reduce confounding factors, such as reproduction and genetic variation that may affect resting metabolism. Furthermore, most studies on energy metabolism usually involve a single measurement at a certain time point; whether or not energy metabolism changes with age within an individual has received little attention (Moe *et al.* 2009). Here, we also investigate the changes of resting metabolism within individuals as they age.

One can think of lifespan as the timeframe in which an organism can reproduce and/or contribute toward its inclusive fitness. Lifespan becomes a very important life history trait for organisms that do not reproduce directly but acquire fitness indirectly. Social insect workers are examples of animals that are non-reproducing and acquire fitness indirectly through colony level reproduction (Hölldobler & Wilson 2009). Social insect colonies have a reproductive division of labor in which mating is left to the queen and males, and the queen is the only female who lays fertilized eggs while the majority of the colony's population is comprised of highly related non-reproducing workers who perform tasks necessary for colony growth and survival. Queens and males are analogous to gametic cells while the workers are analogous to somatic cells of whole organisms. Because social insect colonies as a whole have physiological properties similar to whole organisms, they are often referred to as 'superorganisms' (Hölldobler & Wilson 2009a). Because social insect colonies are comprised mostly of non-reproducing workers whose collective work efforts promote colony fitness, understanding the factors that affect social insect workers' lifespans has been of particular interest (Schmid-Hempel & Wolf 1988). This is because the amount of energy involved in producing a worker is a cost to the colony; however, the colony benefits from producing the worker through the tasks and

overall work output that it contributes to the colony over its entire lifetime. Thus, the longer a worker lives, the more benefits its colony should receive.

If energy metabolism is related to lifespan, what factors affect an individual's metabolism? Body size is a major life history trait that is associated with resting metabolism such that although larger organisms have larger metabolic outputs, they generally have lower mass-specific metabolic rates and longer lifespans than smaller organisms (Kleiber 1932; Brody 1945; Speakman 2005). Thus, body size must be accounted for when studying the relationship between metabolism and lifespan. Reproduction has major metabolic costs (Reznick, Nunney & Tessier 2000; Zera & Harshman 2001; Harshman & Zera 2007) that could lead to trade-offs between traits such as fecundity and longevity (Zera & Harshman 2001). Because social insect workers are non-reproducing, they provide a naturally occurring opportunity to test the relationship between metabolic rate and lifespan without the confounding and significant effects of reproduction. Genetics may also affect resting metabolic rate (Burton *et al.* 2011). Certain social insects have colonies with high genetic relatedness and thus low genetic differences among individuals. Social insects in the Hymenopteran order have a haplodiploid mode of sex determination in which males develop from unfertilized eggs (haploid) and females develop from fertilized eggs (diploid); as a consequence, female workers have high genetic relatedness and share an average of 75% of their genes (Hölldobler & Wilson 2009). This high coefficient of relatedness is shared among all sisters within a single colony if there is one monandrous queen. While colonies of some social insect groups may have a single polyandrous queen (e.g. honey bees) or even multiple queens (e.g. some ants), bumble bee (*Bombus* spp.) colonies develop from a single queen who typically mates once, resulting in highly genetically related workers within the same colony (Estoup *et al.* 1995; Schmid-Hempel & Schmid-Hempel 2000). Furthermore, it is important to reduce extrinsic mortality risk factors (e.g. predation or extreme weather changes) in order to truly study the relationship between resting metabolic rate and lifespan. Because bumble bee colonies can be completely maintained under laboratory conditions, one can study lifespan without extrinsic mortality factors (e.g. predation and extreme weather changes) (Ricklefs & Scheuerlein 2001). We define

‘potential lifespan’ as an individual’s lifespan in the absence of such extrinsic mortality risk factors.

Most studies on energy metabolism involve a single measurement and assume that the measured resting metabolic rate is indicative of the organism throughout ontogeny. However, the effects of age on metabolic rate has received little attention, leaving this assumption largely untested (Sohal 1986; Moe *et al.* 2009). Since social insect workers live in a stationary nest which they always return to, they are easily tracked from birth to death and repeated measures of metabolic rate at various age increments can be accomplished. Thus, the biology of *B. impatiens* reduces the confounding effects of genetic variation and reproduction on metabolic rate, and we can easily measure the relationship of age and resting metabolic rate. Specifically, we ask (1) whether metabolic rate affects potential lifespan among individuals, and (2) whether metabolic rate changes with age within individuals.

Results

Metabolic rate and potential lifespan

The average worker lifespan was 38.3 ± 1.47 days. Mass specific resting metabolic rate negatively correlated with potential lifespan (simple linear regression: log resting mass-specific metabolic rate, $b = -8.83$, $F_{1,32} = 6.00$, $P = 0.0199$, $R^2 = 0.16$, $N = 34$; Fig. 1). Body size, as measured by thorax width (simple linear regression: $F_{1,32} = 0.0099$, $P = 0.92$) or body mass (simple linear regression: $F_{1,32} = 0.31$, $P = 0.58$), was not significantly correlated with worker lifespan (Fig. 2). Body mass and mass specific resting metabolic rate were also not correlated (simple linear regression: $F_{1,32} = 2.01$, $P = 0.17$).

Metabolic rate and age

Overall, there was a significant negative relationship between resting mass-specific metabolic rate and age (Fig. 3A) as well as a significant ‘bee X age interaction’ (Fig. 3B) (multiple linear regression, with ‘bee’ specified as a random factor: age, $b = -0.0060$, $F_{1,270.1} = 22.4$, $P < 0.0001$; bee x age, $F_{49,292.8} = 1.48$, $P = 0.028$, $R^2 = 0.28$, $N = 50$). Because bumble bee workers vary greatly in size (Goulson 2003b), we analyzed whether body size influenced the significant ‘bee X age’ interaction observed in the prior

analysis. Bees ($N = 50$) were divided into four body size categories based on quartiles of their thorax widths. The body size categories were: extra large (4.31 mm – 5.82 mm), large (4.18 mm – 4.30 mm), medium (3.89 mm – 4.17 mm), and small (3.12 mm – 3.88 mm). There was not a significant ‘size X age’ interaction (multiple linear regression, where ‘bee’ is a random factor: age, $F_{1, 381} = 17.7$, $P < 0.0001$; size, $F_{3, 37.92} = 4.01$, $P = 0.014$; size X age, $F_{3, 380.5} = 1.99$, $P = 0.12$). Thus, worker size does not affect the significant ‘bee X age’ interaction observed.

Because bees died at different ages, the number of repeated measures of metabolic rates varied (Table 1). We therefore tested whether the number of repeated measures affected our analysis of how mass specific resting metabolic rate changed with age. This analysis only included bees with mass specific metabolic rates measured on the day of emergence and with known potential lifespan ($N = 34$). We standardized the repeated measures for each bee by picking three age points when metabolic rate was measured: at emergence, mid-life, and last measurement before death. With this subset of bees ($N = 34$), we still observed a significant negative relationship between mass specific resting metabolic rate and age (multiple linear regression, where ‘bee’ is a random factor: age, $b = -0.086$, $F_{1, 66.42} = 4.11$, $P = 0.0467$).

Lastly, we wanted to know if workers’ body masses changed with age, as weight loss or weight gain could affect resting metabolic rate as bees aged. To do this, we analyzed whether body mass changes with age. We found a significant negative correlation between body mass and age, as well as a significant ‘age X bee’ interaction (multiple linear regression, where ‘bee’ was specified as a random factor: age, $b = -0.0006$, $F_{1, 309.4} = 23.30$, $P < 0.0001$; age X bee, $F_{50, 311.7} = 1.47$, $P = 0.029$; $R^2 = 0.88$). The significant ‘age X bee’ interaction indicates that although there was a significant negative correlation between body mass and age, the relationship between these two variables varied among bees.

Discussion

Overall, this study shows that bumble bee workers (*B. impatiens*) with higher mass-specific resting metabolic rates have reduced potential lifespans, which we defined as lifespan in the absence of external mortality (e.g. predation). We also show that mass-

specific resting metabolic rates and body mass generally decrease with age. There are three novel aspects of our study. First, using the bumble bee (*B. impatiens*) as our study organism allowed us to naturally reduce and account for effects that reproduction, genetic variation, and body size could have on metabolic rates. Second, our study design and results suggest that metabolic rate is at least one intrinsic factor that is correlated with lifespan. In general, the lifespan of an individual is affected by both extrinsic and intrinsic mortality factors (Li & Anderson 2013). Because our study was conducted under laboratory conditions where there was no predation, extreme weather, or starvation, effects of extrinsic factors on the lifespan of bumble bee workers in our study were minimized (Ricklefs & Scheuerlein 2001), allowing us to better understand factors that could affect potential lifespan. Third, this study is one of the few which have investigated changes of resting metabolic rate over time using a longitudinal approach, where metabolic rates were repeatedly measured within individuals as they aged. Most studies of metabolic aging use single measurements, in which case it is assumed that the measured metabolic rate remains constant throughout an organism's ontogeny, or are cross-sectional in nature (Moe *et al.* 2009), where metabolic rates are measured from age cohorts. The longitudinal approach with repeated measures that we use in this study reduces variation among individuals of different cohorts.

The rate of living theory proposes that metabolic rate is inversely related to lifespan (Pearl 1928; Beckman & Ames 1998; Speakman 2005; Melvin, Van Voorhies & Ballard 2007). Initial support for the rate of living theory was found by Kleiber (1932) and Brody (1945), who showed patterns in which larger organisms had lower metabolism per gram of tissue compared to smaller organisms when in a fasted and resting state. Metabolic rates scale as M^b , where M is body mass and b is the scaling exponent (West, Brown & Enquist 1997). The scaling exponent between body mass and mass specific resting metabolic rate has been reported to be between -0.2 and -0.33 (Speakman 2005). In addition, larger animals generally live longer than smaller animals, and the scaling relationship between body mass and lifespan has been reported to be between 0.15 and 0.3 (Speakman 2005). Based on these allometries, it is hypothesized that energy metabolism affects lifespan, specifically that animals with lower mass specific metabolic rates have longer lifespans. A mechanism that may explain the rate of living theory

proposed in the 1950s is the free-radical/oxidative stress theory of aging (Harman 1956; Beckman & Ames 1998), which posits that the accumulation of reactive oxygen species (ROS) and other free radicals, which are the byproducts of metabolism, leads to cellular damage, enhanced aging and a shorter lifespan.

Support for the rate of living and radical/oxidative stress theories is, however, inconsistent, especially in intraspecific studies (Speakman 2005). For example, in a study by Speakman et al. (2004), there was a positive association between metabolic rate and lifespan in a cohort of mice. The mechanism for this positive correlation was that mice having higher metabolic rates also had significantly more uncoupling, which is the presence of proton leak pathways that reduce the potential of the inner mitochondrial membrane, and hence, reduce the movement of electrons across the membrane. The reduction of electron movement across the inner mitochondrial membrane thereby reduces harmful ROS production in the mitochondrial membranes and can consequently increase lifespan (Speakman *et al.* 2004). Other studies have reported no correlation between metabolic rate and lifespan (Speakman 2005), such as in *Drosophila* (Hulbert *et al.* 2004) and the blowfly *Calliphora stygia* (Hulbert, Usher & Wallman 2004). The fact that there are inconsistent associations between metabolic rate and lifespan in the literature suggest that lifespan may be *partly* affected by metabolic rate (Orzack 2003), and it is possible that under certain conditions it may play a larger role than other variables. Nevertheless, the results from our study provide support for the rate of living theory, and it is likely that our ability to detect this negative relationship is due to the fact that extrinsic factors of mortality were minimized in our laboratory setting and by using sterile and highly related social insect workers, we were able to exclude the confounding effects of reproduction and reduce genetic variation.

Because we found a negative relationship between metabolic rate and lifespan in this study, it would be expected that bumble bee workers with the lower mass-specific metabolic rates and longer potential lifespans would be larger in body size than those with higher mass-specific metabolic rates and shorter potential lifespans. However, we did not observe any associations between body size (measured either as body mass or thorax width) and lifespan. We also did not observe an association between body size and metabolic rate. The body masses of workers in our study sample (N = 34) ranged from

0.064 grams to 0.22 grams, a 3.4 fold difference. If there is an effect of body size on mass-specific metabolic rate and lifespan in worker bumble bees, then perhaps it is not robust enough to detect with the range of body mass in this study. The variation in mass-specific resting metabolic rate and lifespan of bumble bee workers in this study may be explained by other factors such as the types of tasks that workers perform. It is also possible that under natural conditions, where foragers may fly longer distances and expend more energy, one might see effects of body size on mass-specific metabolic rate and lifespan.

Bodily wear, amount of physical activity, and the age at which individuals engage in certain tasks may affect worker lifespan. For example, mortality in *Bombus melanopygus* Nylander was positively associated with natural wing wear and wing margins that were clipped (Cartar 1992). Wing wear results from collisions with vegetation while foraging. Wing wear may be related to mortality because it may cause increased energy expenditure and less maneuverability (Cartar 1992; Foster & Cartar 2011). It is also possible that work load or work effort could negatively affect a worker's lifespan. Previous work in honey bees (Schmid-Hempel & Wolf 1988a) suggested a possible inverse relationship between workload and lifespan, while it has been demonstrated in house flies (Sohal & Buchan 1981) that there is a negative relationship between physical activity and lifespan. Increased physical activity may negatively affect lifespan through increased metabolic rates and rate of aging (Sohal & Buchan 1981). In honey bees, workers that initiate foraging at earlier ages die earlier (Rueppell *et al.* 2007; Rueppell, Kaftanoglu & Page 2009), and reduction of mortality hazards or foraging efforts do not have strong effects of prolonging life which suggests that transition from non-foraging tasks to foraging itself has strong effects on honey bee worker lifespan (Rueppell *et al.* 2007). Physical activity of the bumble bee workers in this study was not measured, but it is possible that the variation of metabolic rates observed in this study may have been associated with bees' work efforts and types of tasks that they performed.

While there is more interest in understanding the effects of metabolism on lifespan, research on how energy metabolism changes with age is still scarce (Broggi *et al.* 2007; Moe *et al.* 2009). Quantifying the relationship between energy metabolism and age will provide new insights on the physiological factors that influence lifespan.

Although basal metabolic rate changes with age in humans (Roberts & Rosenberg 2006), birds (Broggi *et al.* 2007), and dogs (Speakman, van Acker & Harper 2003; Moe *et al.* 2009), these studies were conducted on representative groups at various time points (Moe *et al.* 2009). A longitudinal approach with repeated measures such as was done here, provides a more accurate description of how energy metabolism changes with age because of the advantage in eliminating biases due to disproportional mortality and inter-individual variation. Our results show that resting metabolic rate generally decreases with age and the results are consistent with previous studies (Speakman, van Acker & Harper 2003; Roberts & Rosenberg 2006; Broggi *et al.* 2007; Moe *et al.* 2009). However, our results also showed significant interindividual differences (Fig. 3B). A decrease in the ability to maintain a constant metabolic rate may occur because of a loss in lean body mass and organ mass (Hughes *et al.* 1998; Even *et al.* 2001; Roberts & Rosenberg 2006). Bees in our study lost weight as they aged, and this may have influenced the observed decrease in resting metabolic rate with age. However, we do not know the factors that contributed to the observed weight loss over time, i.e., loss of lipids, muscle mass, or water content. We also observed a significant ‘age X bee’ interaction, which indicates the presence of interindividual differences in the association between body mass and age. Alternatively, metabolic aging may result from adaptive physiological changes that reduces reactive oxygen species with age (Moe *et al.* 2009).

In summary, the negative correlation between resting metabolic rate and potential lifespan in bumble bee workers found in this study lends support for the rate of living theory. The decrease in resting metabolic rate with age shows evidence for metabolic aging in which maintenance costs decrease as workers become older; this highlights the importance of accounting for age when measuring resting metabolic rates. Studies on social insects, where reproduction and genetic variation can be easily controlled for, may provide unique insights on how selection drives behavioral and physiological traits that directly affects senescence, group performance, and fitness in social insects.

Methods

Bumble bee colonies and maintenance

Colonies of *Bombus impatiens* were purchased from Koppert Biological Systems (Romulus, MI, USA.) in 2009 and maintained in plastic (25 cm x 18 cm x 10 cm) or wooden (38 cm x 23 cm x 8 cm) nest boxes covered with transparent Plexiglas. All nest boxes were connected to separate foraging arenas. The colonies were provided with 50% sugar solution and pollen (Koppert Biological Systems, Romulus, MI, USA) *ad libitum* daily. Colonies were kept under laboratory conditions (10: 14 light: dark cycle; 25 ± 1 °C; ~ 25% humidity) at the University of Arizona in Tucson, AZ

Colonies were checked daily for newly emerged workers (callows). Upon emergence, callows were marked with unique numbered tags for identification and tracking. After tagging, thorax widths were measured with a digital caliper to the nearest 0.1 mm (Neiko Tools, USA). Thorax width is a standard measure of body size in bumble bees (Goulson 2003b; Jandt & Dornhaus 2009b; Jandt, Huang & Dornhaus 2009b; Duong & Dornhaus 2012).

Respirometry experiments

The resting metabolic rate of individual workers was quantified as CO₂ production (VCO₂) at 25 ± 1 °C and ~25% humidity, in a dark room when at rest. For this study, a resting bee was defined as an immobile bee with all her legs touching the floor of the chamber. Measurements were made between 10 AM and 7 PM. To measure resting metabolic rate, a worker was placed in a respirometry chamber (870 ml). Using an air pump (Rena Air 200-ES, Rena, Charlotte, NC, USA), air was circulated from the chamber (at a flow rate of 1000 ml min⁻¹) into a CO₂ gas infrared analyzer (LI-820, LI-COR, Lincoln, NE, USA) and back into the chamber. Using LI-COR software (820-500 V 1.0.0), readings of CO₂ concentration (in parts per million) were recorded at 1 second intervals. VCO₂ (in µg CO₂ s⁻¹) was calculated using the linear relationship dCO₂/dt. A worker's mass specific metabolic rate was calculated as the worker's resting metabolic rate divided by its wet mass measured to the nearest 0.01 mg using an analytical balance (Ohaus Analytical Plus AP2500, Ohaus, Pine Brook, NJ, USA). The wet masses of bees were assessed immediately after respirometry measurements.

Lifespan and aging experiments

In our study, lifespan is defined as the numbers of days bees are alive as adults. To determine if mass specific resting metabolic rate correlates with lifespan, the

metabolic rates of newly emerged workers were measured on the day of emergence ($N = 34$). Newly emerged workers were identified by their significantly lighter coloration from that of older workers (Goulson 2003). Colonies were checked daily for dead bees; their identification was recorded and their lifespan was calculated as the number of days between emergence and death. Two bumble bee colonies were used in this study.

To determine if mass specific resting metabolic rate changes with age, initial mass specific resting metabolic rates were measured on the day of emergence ($N = 41$) or within the first three weeks post emergence ($N = 9$). The mass specific resting metabolic rate of individual bees was then repeatedly measured every 2 - 4 days until the bee died. A single colony was used for this study. Only workers having at least three measures of metabolic rate were used for statistical analysis.

Statistical analyses were conducted using JMP 8.0 (JMP® 1987-2008). Lifespan data were normally distributed (Shapiro-Wilk W test: $W = 0.96$, $P = 0.23$). All statistical analyses of the mass-specific resting metabolic rates were log-transformed to ensure normality of the data. Unless specified, data figures show the non-transformed mass specific resting metabolic rate data. Means are given as ± 1 SE.

List of Symbols and Abbreviations

VCO_2 – Rate of carbon dioxide elimination

CO_2 – carbon dioxide

dCO_2/dt – change in carbon dioxide concentration over time

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Author Contributions

N.C. conceived and wrote the manuscript, developed this study's concept and approach, conducted the experiment and analyses, and created all the figures. T.C. contributed in developing this study's concept and approach and also collected data. G.D. contributed towards data analysis and also provided conceptual ideas for manuscript content. A.D. contributed in developing this study's concept, approach, data analyses, and provided conceptual ideas for manuscript content. All authors provided major contributions in editing this manuscript for submission.

There are no competing interests among any of the authors.

Figures

Figure 1.

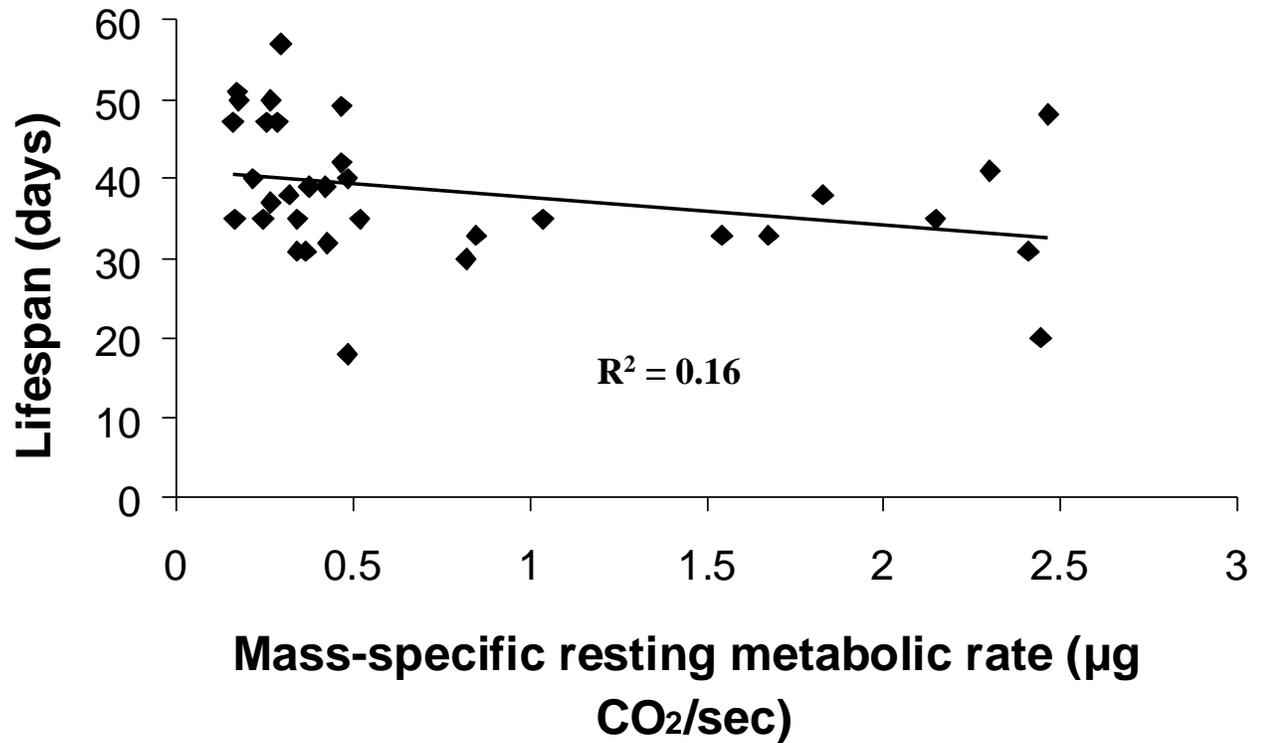


Figure 1. Worker lifespan (days) was significantly and negatively correlated with resting mass-specific metabolic rate. Black diamonds represent individual workers.

Figure 2.

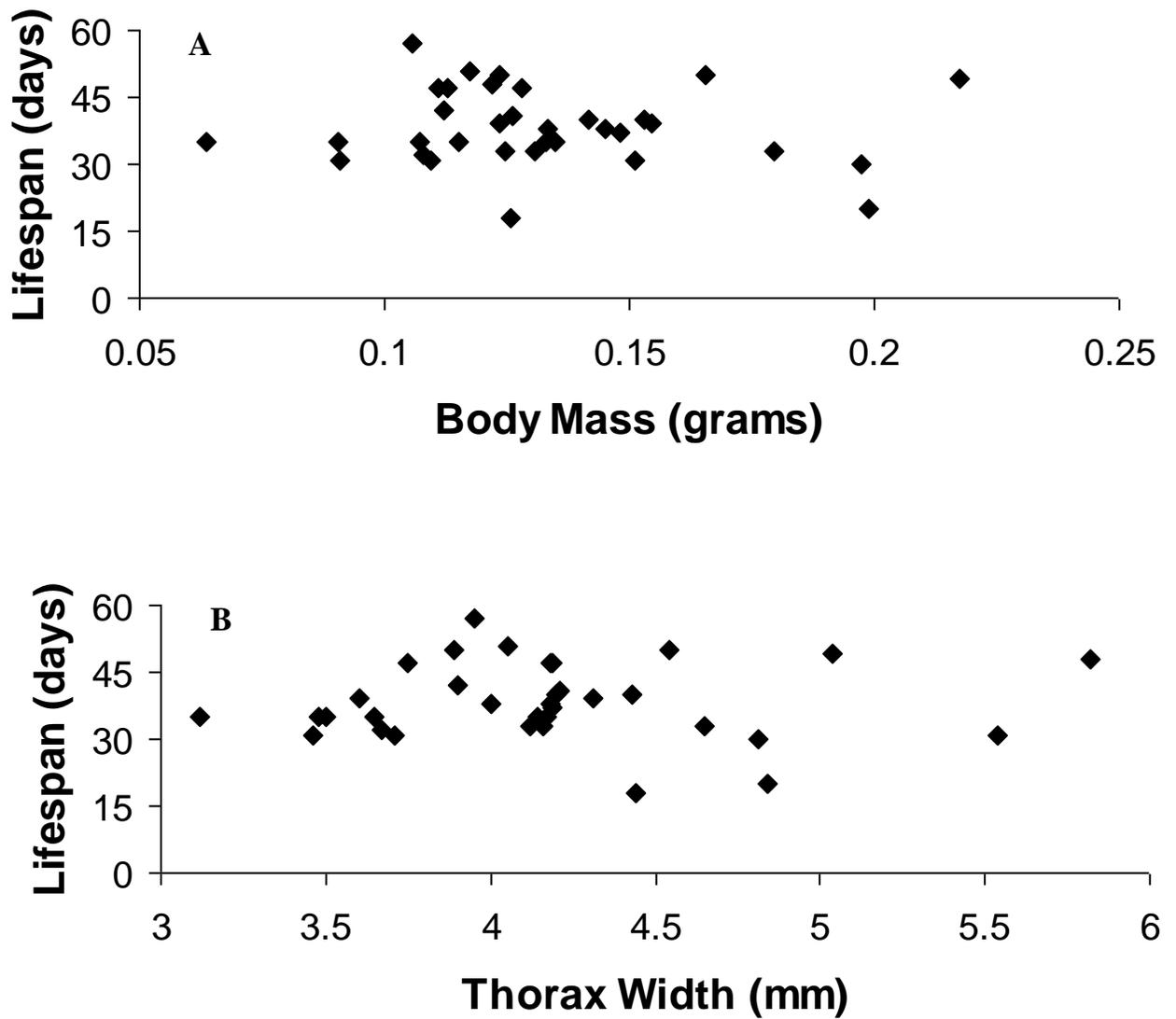


Figure 2. Body size, as measured by body mass (A) and thorax width (B), was not significantly correlated with lifespan.

Figure 3.

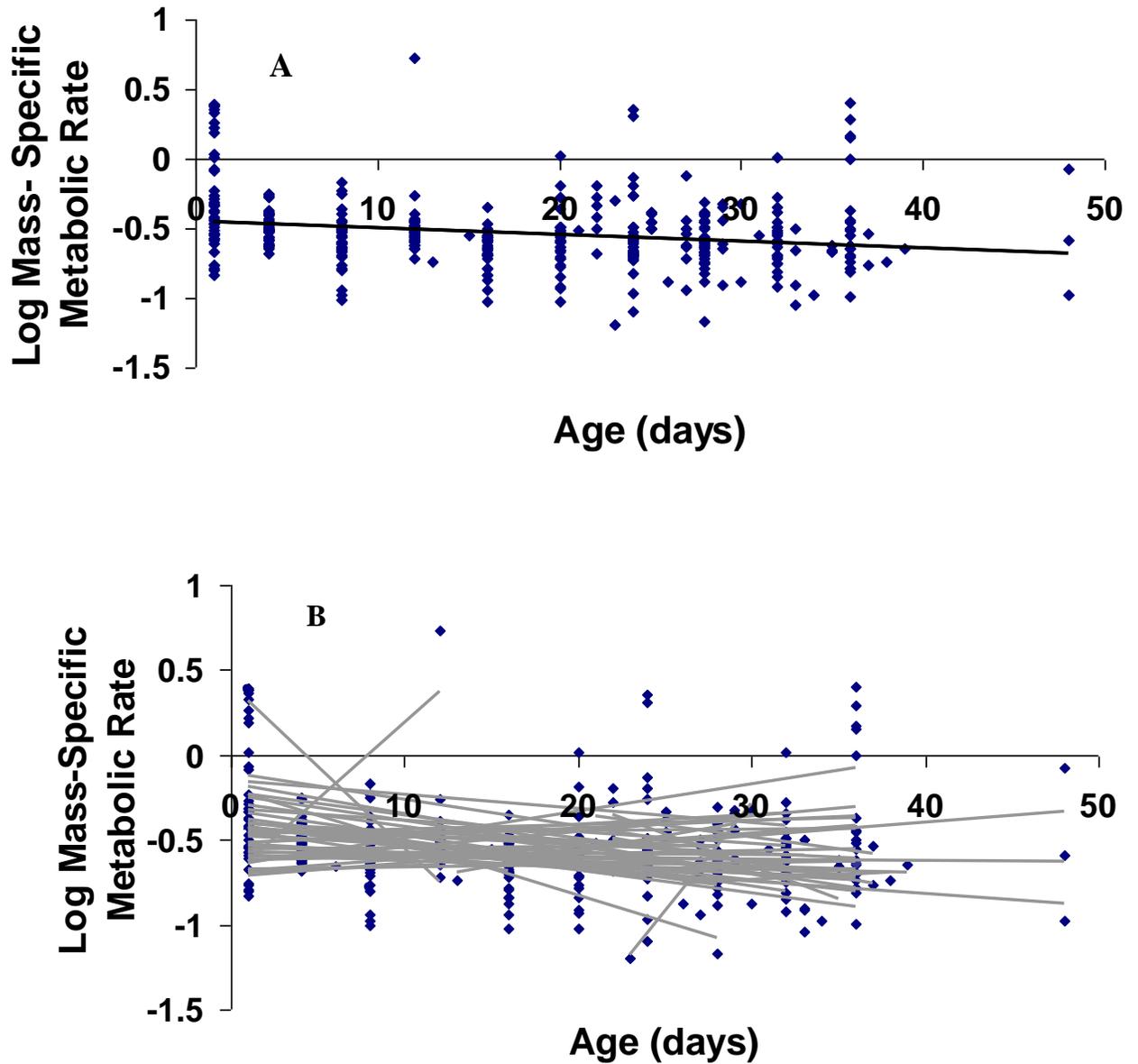


Figure 3. The overall significant decrease in mass-specific resting metabolic rate with age is shown in A and the significant ‘bee X age’ interaction we observed is illustrated by individual bees’ linear regressions in B. Each diamond represents the log mass-specific resting metabolic rate of an individual worker at a particular age.

Table 1.

Number of Bees	Number of Repeated Measures
3	3
4	4
4	5
4	6
5	7
5	8
7	9
14	10
4	11

Table 1. The number of repeated metabolic rate measurements varied for bees, but this did not affect our analyses. Metabolic rate was measured for workers every 2 – 4 days until death. Only bees with at least three measurements were included in our data analyses.

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