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A QUANTITATIVE FRAMEWORK FOR INVESTIGATING LIFE HISTORY TRADE-OFFS IN SOCIAL INSECTS

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ABSTRACT OF THE DISSERTATION

A Quantitative Framework for Investigating Life History Trade-offs in Social Insects

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Life history theory is concerned with understanding the timing of key events in organisms' lives, such as growth, reproduction, and senescence. The evolution of these life history traits often involves trade-offs in the way that organisms allocate their resources. A major goal of life history theory is to understand the selective pressures governing these trade-offs and how they are shaped by organisms' environments. Eusocial organisms, such as the European honeybee, are excellent model systems for exploring life history evolution because of their extraordinary phenotypic plasticity in many relevant life history traits. For organisms with these complex social structures, trade-offs over resource allocation occur at the level of the group rather than the individual. Since most individuals in a eusocial colony do not reproduce, their fitness depends on the success of the colony and selection on life history traits therefore acts mostly on the colony phenotype.

Though there has been much theoretical work on life history evolution, there has thus far been no general framework for understanding the evolution of life history trade-offs in social organisms. To develop such a framework, I create a series of

mathematical models that examine how a eusocial insect colony should optimally allocate energetic resources among survival, growth, and reproduction. I parameterize and test these models using honeybees as a model system and compare model predictions to observed traits in honeybees to gain insight into selective pressures shaping their life history. For my first chapter, I examine how seasonal environmental fluctuations influence the selective pressures on worker senescence in honeybee colonies. For my second chapter, I examine how the costs and benefits associated with worker longevity influence a honeybee colony's optimal investment in worker somatic maintenance. For my third chapter, I explore how trade-offs over resource allocation and sexual selection interact to influence the optimal timing of reproductive investment in honeybee colonies. Together, these models contribute to our understanding of the selective pressures shaping resource allocation in social insect colonies and provide a quantitative framework for examining life history evolution in complex social systems.

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Included in my thesis is a published paper, which is my original work and is co-authored by my advisor, Nina Fefferman.

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INTRODUCTION

One of the overarching principles of evolutionary theory is that selection should favor increased survival and reproduction. Constraints against maximized fitness frequently involve trade-offs between these two critical components because organisms have limited resources to allocate. The field of life history theory seeks to understand how organisms manage these trade-offs and how they are shaped by their ecological environments (Pianka 1970; Stearns 1976; Reznick 2002). The diversity of life histories observed in nature demonstrates how varied the trade-off strategies can be while still achieving minimal evolutionary success (i.e. surviving).

Eusocial organisms, such as the social insects, are fascinating and illuminating systems in which to study life history evolution. Eusociality is a social structure characterized by overlapping generations of related individuals living together, cooperatively caring for brood, and with a division of labor between reproductive and non-reproductive individuals (Wilson and Holldobler 2005). For organisms with these highly complex social structures, many of the basic elements of life history, such as resource acquisition, allocation, and reproduction, occur at the level of the group as well as the individual. While there are large bodies of theory on both life history evolution and the evolution of social complexity, thus far there has been no general quantitative framework for the evolution of life history trade-offs in social systems. In this work, I construct a series of general, abstract mathematical models to provide such a framework. I develop, parameterize and test these models using honeybees as a model

system, asking how social insect colonies should optimally allocate energetic resources among survival, growth, and reproduction and then compare those model results to observed life history traits in honeybees. While there is debate in evolutionary theory over the extent to which evolution optimizes phenotypic traits (Metz et al. 2008), we expect to see general agreement in the observed traits and the model predictions due to the intense selective pressures faced by honeybee colonies during their life cycle (Seeley and Visscher 1985).

Lie history theory

Life history is the sequence and timing of key events in an organism's life related to growth, development, maturity, reproduction, and senescence (Lande 1982). One of the fundamental questions in ecology is why organisms have evolved their various life history strategies. If organisms could optimize all aspects of life history simultaneously, they should all be "Darwinian demons" that mature instantly, have infinite offspring, and live forever (Law 1979). One reason no organisms achieve that hypothetical ideal life history is that all organisms have limited resources and there are trade-offs in how organisms invest these resources. Life history theory seeks to explain broad patterns in how ecological factors influence the way different organisms allocate their resources. These patterns of resource allocation in turn determine most other important aspects of their life history, including how quickly organisms mature, how many times they reproduce, and how long they live (Pianka 1970).

One important element of any organism's life history is their lifespan and the pattern of mortality they experience throughout their lives. Life history theory

sometimes classifies these patterns of mortality as type I (increase in mortality rate with age), type II (constant mortality rate with age) or type III (high juvenile mortality and a decrease in mortality rate with age)(Demetrius 1978). It is usually recognized, however, that actual patterns of mortality rarely fall cleanly into these categories and can take on more complex shapes (Baudisch 2011). At some point in their lives, most organisms experience some level of senescence, a physiological decline in function accompanied by a decrease in survival and/or fertility with age (Medawar 1952). How organisms allocate resources, and particularly how much they invest in somatic maintenance, will influence how quickly they grow, how long they live, and the pattern and rate of senescence they experience.

Another major element of an organism's life history is their timing and level of investment in reproduction. One of the earliest insights of life history theory is that there is often a trade-off between quality and quantity of offspring. Theory predicts organisms selected for fast growth (r strategists) should have more low quality offspring, while organisms selected for competition (k strategists) should have fewer high quality offspring (Pianka 1970). More recently, there has been acknowledgement that many organisms do not fit into this binary categorization and that the selection for optimal reproductive investment can be more complex (Reznick 2002). In addition, the timing of reproduction is important since investing energy in growth at one time might mean more energy for reproduction at some later time. When organisms invest in reproduction and how they allocate that reproductive effort has important consequences for fitness.

Life history in social insects

Eusociality presents a uniquely favorable lens through which to develop and test quantitative models of how trade-offs shape the evolution of life history. Because they have different castes, social insects are characterized by an extreme degree of phenotypic plasticity in genetically similar individuals (Oster and Wilson 1978). This plasticity allows us to separate out genetic effects in examining impacts of changes in resource allocation. In addition, most studies of the relationship between lifespan and reproductive effort are cross-species comparisons (Austad and Fischer 1991; Gaillard et al. 1994; Healy et al. 2014). These studies must be careful to control for phylogeny and it can be difficult to draw causal conclusions because many life history traits (such as body size, developmental rate, and fecundity) are usually highly correlated (Abrams 1993; Reznick et al. 2000). It can therefore be difficult to directly detect trade-offs between survival and reproduction.

Helpfully, social insects allow us to examine different schedules of development, levels of parental investment, and levels of reproductive output within the same species. Social insects also uncouple several life history characteristics that are highly correlated in most other organisms. For instance, social insect queens are both exceptionally long lived among insects (Keller 1998; Keller and Genoud 1997) and characterized by exceptionally high fecundity (Winston 1987). This allows us to make more nuanced investigation into the link between resource investment, survival, and reproductive success.

Another reason that social insects are a useful model system for understanding how organisms allocate resources is that entire colonies can be studied as evolutionary units (Seeley 1997). Whereas in solitary organisms, trade-offs involve the allocation of resources between somatic and reproductive tissue within a single individual, eusocial insect colonies must make decisions about allocation of resources among individuals in the colony (e.g. Crailsheim 1990). While it is usually difficult to directly track the allocation of resources among different tissues in an organism's body, it is much easier to track the allocation of resources among individual insects in a colony. This gives us the unique opportunity to measure the allocation of resources invested in survival and reproduction, rather than simply inferring investment from the resulting level of survival and reproduction.

Social insects and other complex social organisms are therefore interesting models for testing predictions of life history theory, despite the fact that they have been, thus far, predominantly unexploited in this capacity. This work seeks to bridge that theoretical gap by examining the evolution of resource allocation and life history traits at the level of the colony in social insect systems. For my first and second chapters, I examine the evolution of senescence in social insect colonies. For my third chapter, I focus on the evolution of reproductive investment in social insect colonies. Together, these models are intended to provide a quantitative framework to for examining how the major elements of life history (survival, growth, and reproduction) evolve in complex social systems.

Honeybee biology

Though my framework can apply to eusocial insects generally, I focus on honeybees as a particularly good system for studying the evolution of resource allocation. One of two clades of bees to have evolved advanced eusociality (Winston and Michener 1977), honeybee colonies can have up to 100,000 members (Winston 1987). Honeybees have an unusually convex survivorship curve for an insect, making them an interesting system for understanding the role of ecological factors in the evolution of longevity (Sakagami and Fukuda 1968). Most importantly, even among social insects, honeybees have a remarkably plastic aging process, which can be adjusted dynamically according to colony needs (Amdam and Omholt 2002).

While most social insects have drastically different lifespans between workers and queens, honeybees also have a dramatically bimodal and flexible pattern of aging within the worker class (Münch and Amdam 2010). The physiological mechanism of aging in honeybees is reasonably well understood, allowing us to make reasonable assumptions about trade-offs when investigating evolutionary questions (Amdam et al. 2004). Because honeybee colonies can make choices about allocation of resources among workers, and we can measure per worker contributions to colony function, honeybees offer a unique system for investigating the trade-off between current and future reproduction.

The European honeybee, *Apis mellifera*, is native throughout most of Eurasia and Africa and has been introduced to the Americas and Australia (Winston 1987). The species most likely originated in the tropics, but has evolved temperate as well as tropical races (Winston 1987). Unlike most bees, which diapause during the winter,

honeybees are active all year (Seeley and Visscher 1985). However, in temperate regions, honeybees depend on resources available only from spring to fall. Temperate races have evolved a seasonal lifestyle in which they forage and store food during the warmer months and survive winter by actively thermoregulating and relying on stored honey (Winston 1987).

Brood rearing stops in late fall and workers develop into a sub-caste called diutinus bees capable of living much longer than other workers (Omholt and Amdam 2004). In late winter, the colony begins brood rearing again to build up the colony population for spring foraging (Seeley and Visscher 1985). In spring and summer, when floral resources are plentiful, colonies gain weight and increase in size. In late spring or early summer colonies reproduce by swarming (Winston 1987).

During reproductive swarming, the colony begins by rearing a new queen. Before the virgin queen emerges, the mother queen leaves in a swarm with part of the workforce, leaving the daughter queen to inherit the original nest (Winston 1987). The swarm must locate a suitable site and build a new nest. Once the daughter queen emerges, she goes on a mating flight in which she mates with between 7 to 17 males (called drones), on average (Winston 1987). In addition to swarming, a honeybee colony can reproduce by producing drones, which mate with virgin queens from other colonies.

Because of the distinct season of resource availability, ecological constraints on temperate honeybee survival, growth, and reproduction change throughout the year with the result that the timing of life history events is extremely important. They are an excellent model for examining how ecological context shapes selection for resource

allocation in social systems. This work therefore focuses on investigating how seasonally varying constraints on honeybee colonies influence their allocation of resources among growth, survival, and reproduction.

Chapter One

The goal of my first chapter is to apply evolutionary senescence theory to eusocial systems. As social insect workers perform all the survival functions of the colony, they can be thought of as the somatic elements of the colony. Investment in worker longevity is therefore analogous to investment in the maintenance of somatic cells or tissues of an individual organism. The goal of this chapter is to examine the selective pressures that shape individual senescence in a social insect colony.

Senescence theory

Evolutionary aging theory generally agrees that the ultimate cause of senescence is a decline in the force of selection with age (Medawar 1952; Williams 1957; Hamilton 1966). Since even intrinsically immortal organisms are subject to random, extrinsic sources of mortality, as organisms get older, a small fraction of their expected lifetime reproduction remains, resulting in declining reproductive value with age (Medawar 1952).

There are competing, though non-mutually exclusive, ideas of how this decline in selection causes senescence, as well as debate over what ecological factors explain the diverse patterns of senescence among organisms. Mutation accumulation theory suggests the physiological decline is caused by an accumulation of late-acting deleterious mutations not removed by weaker selection later in life (Medawar 1952).

Antagonistic pleiotropy theory suggests that late-acting deleterious mutations can be favored by selection if they have beneficial effects early in life when reproductive value is greater, even if their net effect is neutral or detrimental (Williams 1957). Disposable soma theory suggests that organisms accumulate somatic damage throughout life that if left unrepaired contributes to senescence; since somatic repair is costly, there may be an optimal level of resources to invest in repair that allows some senescence to occur (Kirkwood 1977).

For social insects, workers have little to no direct reproduction, making the concept of remaining lifetime reproductive value meaningless for them. Instead, we would expect the force of selection on worker phenotypes to depend on their remaining lifetime contribution to the colony. In addition, since workers are phenotypically plastic, colonies can respond to changes in the remaining value of workers by adjusting the resources allocated to worker maintenance and their resultant senescence rates. This chapter therefore applies evolutionary aging theory to social systems by exploring how the fitness consequences to a social insect colony of changes in worker senescence are influenced by ecological context.

Senescence in honeybees

The lifespan of honeybee workers has a distinctive seasonal pattern. Summer workers have the shortest lifespans of only 15-38 days, while fall and spring workers live slightly longer (Winston 1987). Winter workers have the longest lifespans and can live up to 10 months (Winston 1987). Longevity is also strongly influenced by behavioral role. Division of labor in honeybee society is based on a system of temporal polytheism

where young workers perform brood care and other in-hive tasks (Winston 1987). As they get older, they transition to storing food, guarding the hive, and then finally foraging (Winston 1987). Workers usually live only 7-10 days once they begin foraging (Münch and Amdam 2010). Workers can postpone foraging depending on colony needs and foragers can sometimes revert back to being hive bees, experiencing a reversal of physiological senescence (Amdam et al. 2005).

The differences in mortality rates between hive bees and foragers are based partly on extrinsic mortality risks and partly on differing rates of senescence. Foragers have a higher risk of extrinsic mortality from exposure to factors such as predators and adverse weather (Dukas 2008). Foragers also have a more rapid rate of physiological senescence than hive bees (Amdam et al. 2004), although if prevented from foraging, hive bees also eventually experience senescence (Remolina et al. 2007).

Seasonal changes in weather and forage availability are important for worker longevity. During winter, when no brood can be reared, worker longevity must increase for the colony to survive until spring and to remain large enough to thermoregulate (Kronenberg and Heller 1982). Swarming also creates a gap in brood rearing because the original queen must stop egg-laying before swarming and the new queen must complete development and mate before she can begin laying eggs (Winston 1987). We therefore expect season and colony life stage to have a large impact on the selection on worker senescence.

In my first chapter, I develop a model of how honeybee colony sensitivity to worker longevity changes under different ecological contexts and compare its

predictions to observed patterns of honeybee worker lifespan and senescence. By developing this model and examining its implications in honeybees, my first chapter gives insights into how ecology shapes the evolution of senescence in a social context.

Chapter Two

While my first chapter explores how we can measure the selective pressures shaping individual lifespan in a social system, my second chapter takes an optimization approach to examine the level of resources a eusocial colony should invest in the maintenance of its workers at various times. While the first chapter examines the fitness benefits of worker longevity, my second chapter additionally integrates the costs of worker maintenance and examines how the trade-off between these costs and benefits influences the lifespan of individuals in a social colony.

Aging and resource investment

The disposable soma theory suggests that the rate of senescence organisms experience depends on the amount of energy invested in somatic repair (Kirkwood 1977). The more energy invested in repair, the less somatic damage accumulates and the slower senescence occurs; however, since organisms have limited resources, the more energy they invest in somatic repair, the less they can invest in reproduction (Kirkwood and Rose 1991). In addition, since most organisms experience random, extrinsic mortality, organisms should only invest enough in repair to produce a soma durable enough to function as long as they are likely to survive (Kirkwood and Austad 2000). Organisms should be expected to optimize this trade-off to maximize their expected lifetime fitness.

In eusocial insects, individual workers have no direct reproductive investment. Instead, selection acts on colonies to optimize the amount of colony resources invested in worker maintenance to maximize the colony's survival and reproduction. For my second chapter, I apply evolutionary aging theory to social systems by examining how a social insect colony's optimal resource allocation determines the lifespan of individual workers. I apply this framework to gain additional insight about the pattern of worker aging in honeybees.

Resource investment in honeybees

Our current understanding of the physiological mechanism of aging in honeybees is consistent with the theoretical prediction that senescence rate and lifespan are influenced by energy and/or nutrient investment. They are therefore a good model for examining how optimal resource investment shapes senescence in social systems. Aging in honeybees is strongly influenced by protein stores; vitellogenin is an important protein storage molecule that has immune functions and promotes longevity (Amdam and Omholt 2002; Amdam et al. 2004; Smedal et al. 2009). Honeybee queens have the largest fat body protein stores and the greatest longevity. Foragers have limited protein stores and have reduced immunity and capability for somatic repair (Münch and Amdam 2010). In spring and summer, nurse bees have higher protein stores than foragers and intermediate lifespans. Winter bees attain the longest lifespan of any worker by storing queen-like quantities of protein (Amdam and Omholt 2002).

Honeybee brood also require protein to develop (Crailsheim 1990). Feeding brood reduces the protein stores of workers by redirecting vitellogenin into brood food

instead of the fat bodies (Amdam and Omholt 2002; Amdam et al. 2009; Smedal et al. 2009). There is therefore a trade-off between colony growth, by producing new workers, and the maintenance of existing workers. This trade-off will be influenced by the risk of extrinsic worker mortality, the efficiency with which protein investment produces gains in worker longevity, and the sensitivity of the colony's growth to worker lifespan. In my second chapter, I develop a model to examine how this trade-off shapes a honeybee colony's optimal level of investment in worker maintenance under different conditions and parts of the colony lifecycle. More broadly, this chapter examines how optimal resource allocation at the colony level shapes individual senescence in social systems.

Chapter Three

While my first two chapters focus on a social insect colony's investment in growth and maintenance, in my third chapter, I examine the evolution of reproductive investment in social systems. The goal of this third chapter is to examine how natural selection has shaped the timing of reproduction and allocation between female and male components of reproductive fitness in social insect colonies.

Reproductive investment

Life history theory suggests that organisms should allocate resources among growth, survival, and reproduction to maximize their fitness. Organisms must decide when to reproduce and how much energy to invest in reproduction. Often the total energy budget is not fixed but is itself influenced by allocation since more energy invested in growth at one time can mean more total energetic resources to allocate

later (van Noordwijk and de Jong 1986; Abrams et al. 1996). Selection therefore should favor the reproductive timing that optimizes the trade-off between acquiring resources and investing them in reproduction.

In addition, organisms must allocate their total reproductive investment among their offspring. This includes trade-offs between the quality and quantity of offspring (Williams 1966; Smith and Fretwell 1974; Mangel et al. 1994). In sexual organisms, there are also trade-offs between the production of males and females. Fisher's sex ratio theory predicts that sexual selection should result in equal investment in male and female offspring (Hamilton 1967). Where males and females have equal costs, the equilibrium strategy should result in an even sex ratio but where there is a cost asymmetry between the sexes, equal investment results more of the cheaper sex (Hamilton 1967).

Reproduction for social insects is not the production of individuals but the production of new colonies. While producing and maintaining workers is an investment in colony growth and survival, producing reproductive individuals (queens and males) that found new colonies is an investment in reproduction. During colony reproduction, honeybees must decide not only when to produce reproductives, but how much to invest in offspring colonies, and how much to invest in daughter queens and males.

Honeybee reproduction

Because of their way of reproducing, the trade-off between growth and reproduction in honeybees is linked to the offspring sex ratio. Honeybee colonies reproduce by fissioning, also called reproductive swarming (Seeley 1995). During

swarming, the colony produces daughter queens that mate and inherit a fraction of the workforce. One daughter also inherits the natal nest, while the mother queen founds a new nest with the rest of the workers. The reproductives are never solitary and cannot survive without workers. The swarm should therefore be considered part of the colony's investment in the female component of reproduction (Bulmer 1983). The other way to pass on the colony's genes is by producing male bees or drones. The drones then compete to be among the 7-17 males who mate with virgin queens from other colonies that are swarming, and found new colonies partially fathered by the drone.

There is a major asymmetry in the costs and benefits of producing male or female reproductives for honeybees. Drones are cheap as they require only the production of a single bee. In contrast, queens are expensive because in addition to the cost of the queen herself, the queen-founded swarm requires the production of thousands of worker bees. Since males are much cheaper than swarms, many more males than females must be produced to result in equal investment between the sexes. Because there are many more drones than queens, each drone has a very low probability of mating, whereas queens have a high chance of successfully mating and founding a daughter colony (Winston 1987). Inclusive fitness theory also predicts that reproductive investment should be discounted by the relatedness to the offspring produced (Trivers and Hare 1976). Because in honeybees there is a difference in the relatedness of workers to drone and queen founded colonies, we expect investment in each sex to be proportional to their relatedness to workers of the parent colony rather than equal.

Because honeybees have a finite season for reproduction to occur, the timing of investment in male (drones) and female (swarms) components of reproduction also determines how much energy the colony will have to invest. Since workers acquire resources for the colony, each worker produced contributes to the rearing of future workers. The opportunity cost of producing a drone includes not only the workers that could be produced with the same amount of resources, but the compounded benefit: the future workers that those workers would have produced. This opportunity cost therefore changes depending on how much time remains in the reproductive season. The timing of male production is therefore influenced by both energetic trade-offs between growth and reproduction and sexual selection on the resources invested in offspring of each sex.

Life history theory makes predictions about how optimal resource allocation shapes reproductive timing and about how sexual selection shapes offspring sex ratios in individuals. In my third chapter, I combine these theoretical frameworks to examine the selective pressures shaping reproductive investment in a social insect that reproduces by colony fission. I develop an optimization model that make predictions about the timing and allocation of reproductive effort in honeybees and compare its predictions to observed honeybee reproductive behavior. The broader goal of this chapter is to provide a framework for examining how seasonality, social organization, and sexual selection influence the evolution of reproductive investment in social systems.

Together these three chapters will build a quantitative framework for examining the trade-offs and selective pressures that shape the evolution of life history traits in social species. They will examine how these trade-offs over resource allocation have shaped honeybee evolution and lay the groundwork for a broader incorporation of selection on social systems into life history theory.

The Sensitivity of a Honeybee Colony to Worker Mortality Depends on Season and Resource Availability

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Abstract

Honeybees have extraordinary phenotypic plasticity in their senescence rate, with seasonal variation in both senescence and extrinsic mortality resulting in up to a tenfold increase in worker life expectancy in winter as compared to summer. To understand the evolution of this remarkable pattern of aging, we must understand how factors affecting the longevity of individuals scale up to effects on the entire colony. Borrowing methods from population demographic modelling, we develop a matrix model of colony demographics to ask how worker age-dependent and age-independent mortality affect colony fitness and how these effects differ by seasonal conditions. We find that there are seasonal differences in honeybee colony sensitivity to both senescent and extrinsic worker mortality. Colonies are most sensitive to extrinsic (age-independent) nurse and forager mortality during periods of higher extrinsic mortality and resource availability

but most sensitive to age-dependent mortality during periods of lower extrinsic mortality and lower resource availability. These results suggest that seasonal changes in selection on worker senescence may partly explaining the observed pattern of seasonal differences in worker aging.

Introduction

One challenge of life history theory is explaining the great diversity of lifespans and patterns of senescence we see in the natural world. Senescence, which can be defined as a decline in physiological functioning that is usually accompanied by an increase in the rate of mortality with age, seems puzzling since natural selection should eliminate traits that reduce survival or fecundity.

The main evolutionary explanation of senescence is a decline in the force of selection with age due to random mortality (Medawar 1952; Hamilton 1966). This decline in the force of selection may cause senescence because of the accumulation of late-acting deleterious mutations (mutation accumulation theory) (Medawar 1952) or positive selection for genes that are beneficial early in life but detrimental later in life (antagonistic pleiotropy theory) (Williams 1957). Kirkwood (1977) proposed that a physiological mechanism for antagonistic pleiotropy may be an energetic cost to somatic maintenance (disposable soma theory). If there is a tradeoff between investment in reproduction and maintenance, selection may favor an optimal level of investment in maintenance that allows some accumulation of damage, resulting in senescence (Kirkwood 1977, 2010; Kirkwood and Rose 1991).

Early proponents of both mutation accumulation and antagonistic pleiotropy theories predicted that increasing the level of extrinsic mortality should accelerate the decline in selection with age, resulting in increased senescence (Medawar 1952; Williams 1957; Hamilton 1966). However, further refinement of these theories has led to debate over how the force of selection changes with age and how extrinsic mortality

affects the force of selection against senescence. Abrams pointed out that in a density-independent population, higher extrinsic mortality doesn't change the force of selection if it is the same across all age groups (Abrams 1993; Caswell 2007). The effect of extrinsic mortality on senescence also depends on the type of density dependence (Abrams 1993).

If a source of extrinsic mortality, such as predation, isn't random with respect to condition, it can increase rather than decrease the selection against senescence (Williams and Day 2003; Chen and Maklakov 2012). In addition, the force of selection may not inevitably decline with age and can even increase (Baudisch 2005) resulting in negligible or negative senescence (Vaupel et al. 2004). These more nuanced theoretical predictions may explain why there has been mixed empirical support for the initial prediction that higher extrinsic mortality causes faster senescence (Promislow and Harvey 1990; Austad 1993; Gaillard et al. 1994; Keller and Genoud 1997; Stearns et al. 2000; Reznick et al. 2004; Williams et al. 2006).

The European honeybee (*Apis mellifera*) is a useful model system for empirically testing predictions about how changes in the force of selection influence the evolution of senescence. Honeybees have a remarkable degree of phenotypic plasticity in the rate of aging within the worker caste, with workers having up to a tenfold difference in life expectancy based on season, social environment, and task performance (Fluri et al. 1977; Amdam and Omholt 2002; Amdam et al. 2005, 2009; Münch and Amdam 2010). Because of their division of labor and seasonally changing environment, we would

expect a large degree of variation in the selective pressure on the senescence of honeybee workers.

Although it is recognized that sociality strongly influences the evolution of senescence (e.g. Lee 2003), there is a relative dearth of theory on factors affecting the force of selection against senescence in eusocial organisms. One challenge of understanding senescence in social organisms is that it can be difficult to know how changes in the longevity of individuals will scale up to effects on the whole colony, the relevant unit of selection (Seeley 1997). Understanding how to estimate the selective pressure against worker senescence in honeybees can thus give us broader insights into the evolution of aging in social systems.

The question we therefore seek to address is how seasonal changes in extrinsic mortality and resource availability influence the selective pressure on worker senescence in honeybees. Using a demographic model, we ask a) how sensitive is colony growth to changes in age-dependent and age-independent worker mortality, b) how does this sensitivity differ by season, and c) do seasonal changes in the force of selection predict the observed pattern of worker senescence?

Honeybee colonies have an age based division of labor in which young workers work inside the hive as nurses and older workers forage outside (Winston 1987). Nurses have a lower senescence rate than foragers (Amdam et al. 2005) and a much lower risk of accidental mortality because of the protected environment of the hive (Münch and Amdam 2010). Worker lifespan also has a distinct seasonal pattern. Summer bees have the shortest lifespans of 2-6 weeks, spring and fall bees have intermediate lifespans, and

winter bees have the longest lifespans of up to 20 weeks (Winston 1987). Honeybees rely on a seasonal food resource and colonies must survive the winter when they are unable to forage or rear brood. Because of seasonal changes in both extrinsic mortality and food availability, we would expect the fitness effects to the colony of changes in worker senescence to vary strongly by season.

To answer our research question, we adapt a method commonly used in demographic modeling: the Leslie matrix model. This framework is typically used to estimate the growth rate of an age-structured population and to examine how different life stages contribute to the growth of a population (Caswell 1989). In conservation, it can be used to determine which life stage to target to have the biggest impact on a population's growth (Caswell 1989).

We adapt this method to model the growth of a social insect colony instead of a population. Since honeybee workers have little or no direct reproduction, their fitness is determined by the reproductive success of their colony (Seeley 1997). We assume that the selective pressure on worker traits is proportional to the effect of the trait value on colony growth and/or survival. We therefore estimate the selective pressure on worker senescence by calculating the sensitivity of colony growth to changes in worker mortality.

This method gives us a computationally simple way to estimate how different worker life stages differ in their contribution to colony growth and how changes in the vital rates of individual workers affect the fitness of the colony.

Methods

We construct an age-structured Leslie matrix model of a honeybee colony. We divide the worker population into brood, nurse, and forager stages, with each stage further divided into age classes. We define $B_{i,t}$ as the number of *i* day old brood in the colony on day *t*, $N_{i,t}$ as the number of *i* day old nurse bees in the colony on day *t*, and $F_{i,t}$ as the number of *i* day old foragers in the colony on day *t*.

Rather than fecundity, as in a traditional Leslie matrix, the top row of the Leslie matrix represents the contribution of each forager to the production of new worker brood. We assume that brood development is limited only by the ability of the colony to feed them (i.e., assuming the colony is not near the queen's egg laying capacity). We assume that workers remain in the brood stage for 21 days (Winston 1987) and brood survive to the next age class with probability s_b. We define *r* as the number of new brood that can be provisioned by a forager per day, with $r = \frac{p}{c}$, where *c* is the amount of food required by a brood per day and *p* is the amount of food provisioned by a forager per day. Thus, the number of *i* day old brood on day *t* is defined by:

$$B_{i,t} = \begin{cases} \sum_{j=1}^{19} rF_{j,t-1} & i = 1 \\ s_b B_{i-1,t-1} & i = 2{:}\,21 \end{cases}$$

We assume all adult workers start as nurse bees and become foragers after a variable number of days (Robinson et al. 1989). We define *g* as the probability a nurse becomes a forager the next day (note that this is different from a deterministic progression to forager after a fixed number of days). We assume nurse bees have a low rate of senescence and a low probability of extrinsic mortality because of the protected

environment of the hive (Rueppell et al. 2007; Dukas 2008). We assume nurse survival is influenced by both senescence (age-dependent mortality) and extrinsic hazards (age-independent mortality) (Dukas 2008). We define $s_{n,i}$ as the daily survival probability of an *i* day old nurse and $m_{n,i}$ as the daily mortality probability of an *i* day old nurse. We represent nurse mortality as a Gompertz-Makeham function where:

$$s_{n,i} = 1 - m_{n,i}$$

$$m_{n,i} = \alpha_n e^{\beta_n i} + \gamma_n$$

We refer to the intercept γ_n as the nurse extrinsic mortality parameter since it represents the age-independent component of nurse mortality. We refer to α_n as the initial age-dependent nurse mortality parameter and to β_n as the age-dependent increase in nurse mortality parameter. We assume that changes in α_n and β_n reflect changes in senescence. The number of *i* day old nurses on day *t* is given by:

$$N_{i,t} = \begin{cases} s_b B_{21,t-1} & i = 1 \\ s_{n,i-1}(1-g)N_{i-1,t-1} & i = 2{:}\,120 \end{cases}$$

We assume, like nurses, forager survival is influenced by both age-dependent mortality and age-independent mortality (Dukas 2008). We define $s_{f,i}$ as the daily survival probability of an *i* day old forager and $m_{f,i}$ as the daily mortality probability of an *i* day old forager. We represent forager mortality as a Gompertz-Makeham function where:

$$s_{f,i} = 1 - m_{f,i}$$

$$m_{f,i} = \alpha_f e^{\beta_f i} + \gamma_f$$

We refer to the intercept γ_f as the forager extrinsic mortality parameter. We refer to α_f as the initial age-dependent forager mortality parameter and to β_f as the age-

dependent increase in forager mortality parameter. As with nurses, we assume α_f and β_f represent forager senescence. We assume all workers go through a nurse stage before becoming foragers. We assume the number of workers living more than 19 days as foragers is negligible (Dukas 2008). The number of *i* day old foragers on day *t* is given by:

$$F_{i,t} = \begin{cases} \sum_{j=1}^{120} s_{n,j} g N_{j,t-1} & i = 1 \\ s_{f,i-1} F_{i-1,t-1} & i = 2{:}\,19 \end{cases}$$

To examine how the selective pressures shaping worker aging differ across annual environmental fluctuations, we modeled a colony under three different seasonal conditions: spring/fall, summer, and winter. We represented each season by different parameter values for forager extrinsic mortality (γ_f), food availability (*p*), and nurse-to-forager transition rate (*g*) (Table 1). We represented summer as a season with high food availability, high extrinsic mortality, and a high nurse-to-forager transition rate. We represented fall and spring as intermediate food availability, intermediate extrinsic mortality, and a high nurse-to-forager transition rate. We represented winter as near zero food availability, low extrinsic mortality, and low nurse-to-forager transition rate since winter bees do not leave the hive to forage.

To examine the effects of forager and nurse extrinsic mortality and senescence on the growth of the colony, we performed a numeric elasticity analysis by perturbation (Caswell 2000). Elasticity is a measure of sensitivity that is scaled to be unitless (Caswell 1989). We calculated the elasticity of the colony growth rate (the dominant eigenvalue of the Leslie matrix) to perturbations in parameters γ_n, α_n, and β_n (the nurse mortality

parameters) and γ_f, α_f, and β_f (the forager mortality parameters). If we define λ as the colony growth rate, the elasticity of the growth rate to parameter x is defined as:

$$\frac{\Delta\lambda}{\Delta x}\frac{x}{\lambda}$$

We repeated this elasticity analysis for each set of seasonal parameter conditions to examine how the selective pressure on worker age-dependent and age-independent mortality differs by season.

Results

We find that the elasticity of the colony growth rate, λ, to the age-independent (extrinsic) component of nurse mortality, γ_n, is highest under summer conditions (high productivity and high extrinsic mortality) and lowest under winter conditions (low productivity and low extrinsic mortality). The elasticity to γ_n under spring/fall conditions (intermediate productivity and extrinsic mortality) is similar to that of summer conditions (Figure 1).

In contrast, we find that the elasticities of the colony growth rate to the age-dependent increase in nurse mortality, β_n, and to the initial age-dependent nurse mortality, α_n, are both highest under winter conditions and lowest under summer and spring/fall conditions (Figures 2 and 3). Taken together, these results suggest that a honeybee colony is most sensitive to changes in nurse senescence during the winter but most sensitive to changes in nurse extrinsic mortality during the summer. Table 2 shows the elasticity of the λ to γ_n, α_n, and β_n in each season.

We further find that the elasticity of the colony growth rate to γ_f, the age-independent (extrinsic) component of forager mortality, is highest under summer

conditions (high productivity and high extrinsic mortality) and lowest under winter conditions (low productivity and low extrinsic mortality). Unlike for nurse mortality, the elasticity of the growth rate to γ_f under fall/spring conditions is intermediate between that of summer and winter (Figure 4). This suggests that the selective pressures against extrinsic forager mortality, like extrinsic nurse mortality, are strongest in summer and weakest in winter.

In contrast, we find that the elasticity of λ to α_f, the initial age-dependent forager mortality, is highest under winter conditions, intermediate under spring/fall conditions, and lowest under summer conditions (Figure 5). Similarly, we find that the elasticity of λ to β_f, the age-dependent increase in forager mortality, is highest under winter conditions and similarly low under summer and fall/spring conditions, although it is lowest in summer conditions (Figure 6). Together this suggests that the selective pressures against forager senescence are strongest in winter and weakest in summer. Table 3 shows the elasticity of the λ to γ_f, α_f, and β_f in each season.

Discussion

Much of our evolutionary understanding of senescence is based on the principle that organisms experience a decline in the force of selection with age (Medawar 1952; Hamilton 1966) resulting in positive selection for traits that increase early-life survival or fecundity at the expense of late-life survival (Williams 1957). Theory further predicts that investing in somatic maintenance to postpone senescence is energetically costly (Kirkwood 1977); when selection declines more rapidly with age, organisms should invest less in somatic maintenance and experience more rapid senescence. Differences

in mean longevity and senescence rate among organisms should therefore be explained at least in part by differences in the pattern and degree to which selection changes with age.

Social insects, such as honeybees, are excellent model systems for exploring the evolution of senescence because of their large degree of phenotypic plasticity in senescence rate and lifespan among genetically similar individuals (Keller and Genoud 1997; Page and Peng 2001; Münch and Amdam 2010). Different workers experience different levels of extrinsic hazards depending on their behavioral role in the colony (Dukas 2008). In addition, extrinsic mortality, resource availability, and worker behavior vary seasonally, allowing us to examine how senescence in workers is influenced by ecological context.

There has been much theoretical work refining predictions about how extrinsic mortality (Cichoń 1997), density-dependence (Abrams 1993), and other ecological factors (Williams and Day 2003) affect the selection against senescence in individuals. However, it is less straightforward how these ecological factors influence the strength of selection against senescence in social organisms, where individuals have little or no direct reproduction and fitness depends on their contribution to the colony as a whole. Using a simple stage-structured demographic model, we seek to bridge this theoretical gap to explore how ecological context influences selection against worker senescence in honeybees.

We find that there are seasonal differences in the strength of selection against senescence in honeybee workers, as measured by the sensitivity of the colony growth

rate to age-dependent worker mortality. We find that the colony is more sensitive to changes in both nurse and forager senescence in winter conditions, when resources are scarce and extrinsic mortality is lower, than in summer conditions, when resources are plentiful and extrinsic mortality is high (Figures 3 and 6). Since colonies cannot easily produce new workers in winter, small increases in the senescence of existing workers have larger effects on the colony. This difference in sensitivity may largely explain why winter honeybee workers have a much lower senescence rate than spring or summer workers (Münch et al. 2013). In contrast, colonies are most sensitive to changes in extrinsic mortality (Figures 1 and 4) in summer when resources are plentiful; this may be because summer workers spend more of their lives in the riskier forager state rather than the more protected nurse state (Winston 1987).

We also find the seasonal pattern of selection changes with worker life stage. There is much stronger selection against nurse senescence in winter, when most workers remain in the nurse stage, than in summer and spring/fall, both periods when they are likely to transition into foragers sooner (Figure 3). Since nurses have much lower age-dependent and -independent mortality than foragers, selection against nurse senescence in summer is driven partly by how quickly they transition to the riskier forager state. The selection against foragers senescence, on the other hand, is strongest in winter, but intermediate in spring/fall and lowest in summer (Figure 6), suggesting that selection on forager senescence decreases as extrinsic mortality increases. This aspect of our results highlights how behavioral role can interact with ecological context to influence how the selection against senescence changes with age.

Overall, our model predicts that the selection against worker senescence should be strongest in winter and weakest in summer. This should lead to the evolution of seasonal differences in worker senescence rate, with the slowest senescence in winter and the fastest in summer. This prediction about the seasonal pattern of senescence rate matches what we observe empirically in temporal honeybee colonies (Sakagami and Fukuda 1968; Münch and Amdam 2010; Münch et al. 2013). This model therefore suggests that seasonal changes in the force of selection are important in shaping the phenotypically plastic pattern of senescence in honeybees.

Although the main objective of this model is to estimate how seasonally varying selective pressures affect the evolution of aging in honeybee workers, this method could also be used to predict how anthropogenic sources of mortality will affect the health and survival of honeybee colonies. The European honeybee is an economically important pollinator, whose crop pollination services are worth an estimated at $11.68 billion annually in the United States (Calderone 2012). Managed honeybees face numerous stressors including parasites, nutrition stress, and pesticide exposure. Because of logistical constraints, the impact of potential threats to honeybee health are usually evaluated at the individual rather than colony level (USEPA 2012). This model can therefore help predict how changes in individual worker mortality will scale up to colony-level effects, which is important to evaluating threats to honeybee health and also can give clues to the causes of colony declines (Khoury et al. 2013; Perry et al. 2015).

The principle that selection changes with age has been a cornerstone of much of evolutionary senescence theory (Medawar 1952; Williams 1957; Hamilton 1966; Abrams 1993; Baudisch 2005; Kirkwood 2010). There has been much interest in refining our understanding of how ecological factors, such as extrinsic hazards, influence the age-specific patterns of selection and in turn the evolution of lifespan. Previous work has shown that the force of selection doesn't simply decline linearly with age, but can have more complex patterns (Abrams 1993; Vaupel et al. 2004; Baudisch 2005, 2011). We here demonstrate how seasonal changes in the strength of selection can explain phenotypically plastic differences in lifespan among individuals in a social species. This simple approach to quantifying the effect of worker mortality on colony fitness can lead to better empirical predictions about how ecological factors should influence the evolution of senescence in social organisms.

Tables

Table 1. Full list of model parameters and their values.

Parameter	Definition	Summer	Fall/Spring	Winter
p	Food production per forager per day	0.098	.058	0.0001
c	Total food consumption per brood	0.151	0.151	0.151
r	Number of brood provisioned per forager per day	p/c	p/c	p/c
s_b	Daily brood survival probability	0.993	0.993	0.993
α_n	Nurse initial age-dependent mortality rate	0.001	0.001	0.001
β_n	Nurse age-dependent increase in mortality rate	0.1	0.1	0.1
γ_n	Nurse age-independent mortality rate	0.04	0.04	0.0134
α_f	Forager initial age-dependent mortality rate	0.001	0.001	0.001
β_f	Forager age-dependent increase in mortality rate	0.369	0.369	0.369
γ_f	Forager age-independent mortality rate	0.134	0.067	0.0134
g	Nurse probability of becoming forager per day	0.05	0.05	0.01
T	Number of days considered	90	90	90

Table 2. Elasticities of colony growth rate to nurse mortality parameters by season

	$\boldsymbol{\alpha_n}$	$\boldsymbol{\beta_n}$	$\boldsymbol{\gamma_n}$
Spring/Fall	0.000911	0.00206	0.01026
Summer	0.000886	0.00195	0.01039
Winter	0.011243	0.04413	0.00759

Table 3. Elasticities of colony growth rate to forager mortality parameters by season

	$\boldsymbol{\alpha_f}$	$\boldsymbol{\beta_f}$	$\boldsymbol{\gamma_f}$
Spring/Fall	0.00358	0.01490	0.01107
Summer	0.00358	0.00574	0.01971
Winter	0.00685	0.12545	0.00186

Figure Legends

Figure 1. Elasticity of λ to γ_n, the age-independent component of nurse mortality. Nurse mortality is represented as a Gompertz-Makeham function, with γ_n as the intercept or age-independent component of nurse mortality. Colonies are less sensitive to nurse age-independent mortality in winter (low extrinsic mortality, low forager productivity, low forager transition rate) than in summer (high extrinsic mortality, high forager productivity, high forager transition rate) or spring (intermediate extrinsic mortality, intermediate forager productivity, high forager transition rate).

Figure 2. Elasticity of λ to α_n, the initial nurse mortality. Nurse mortality is represented as a Gompertz-Makeham function, with α_n as the initial age-dependent component of nurse mortality. Colonies are more sensitive to nurse age-dependent mortality in winter (low extrinsic mortality, low forager productivity, low forager transition rate) than in summer (high extrinsic mortality, high forager productivity, high forager transition rate) or spring (intermediate extrinsic mortality, intermediate forager productivity, high forager transition rate).

Figure 3. Elasticity of λ to β_n, the age-dependent increase in nurse mortality. Nurse mortality is represented as a Gompertz-Makeham function, with β_n as the exponential rate of increase in nurse mortality with age. Colonies are much more sensitive to the age-dependent increase in nurse mortality in winter (low extrinsic mortality, low forager productivity, low forager transition rate) than in summer (high extrinsic mortality, high

forager productivity, high forager transition rate) or spring (intermediate extrinsic mortality, intermediate forager productivity, high forager transition rate).

Figure 4. Elasticity of λ to γ_f, the age-independent component of forager mortality. Forager mortality is represented as a Gompertz-Makeham function, with γ_f as the intercept or age-independent component of forager mortality. Colonies are least sensitive to forager age-independent mortality in winter (low extrinsic mortality, low forager productivity, low forager transition rate) and most sensitive in summer (high extrinsic mortality, high forager productivity, high forager transition rate), with spring/fall (intermediate extrinsic mortality, intermediate forager productivity, high forager transition rate) elasticity being intermediate between that of summer and winter.

Figure 5. Elasticity of λ to α_f, the initial forager mortality. Forager mortality is represented as a Gompertz-Makeham function, with α_f as the initial age-dependent component of forager mortality. Colonies are most sensitive to forager age-dependent mortality in winter (low extrinsic mortality, low forager productivity, low forager transition rate) and least sensitive in summer (high extrinsic mortality, high forager productivity, high forager transition rate), with spring/fall (intermediate extrinsic mortality, intermediate forager productivity, high forager transition rate) elasticity being intermediate between that of summer and winter.

Figure 6. Elasticity of λ to β_f, the age-dependent increase in forager mortality. Forager mortality is represented as a Gompertz-Makeham function, with β_f as the exponential

rate of increase in forager mortality with age. Colonies are most sensitive to the age-dependent increase in forager mortality in winter (low extrinsic mortality, low forager productivity, low forager transition rate) and least sensitive in summer (high extrinsic mortality, high forager productivity, high forager transition rate), with spring/fall (intermediate extrinsic mortality, intermediate forager productivity, high forager transition rate) elasticity being intermediate but closer to that of summer.

Figures

Figure 1

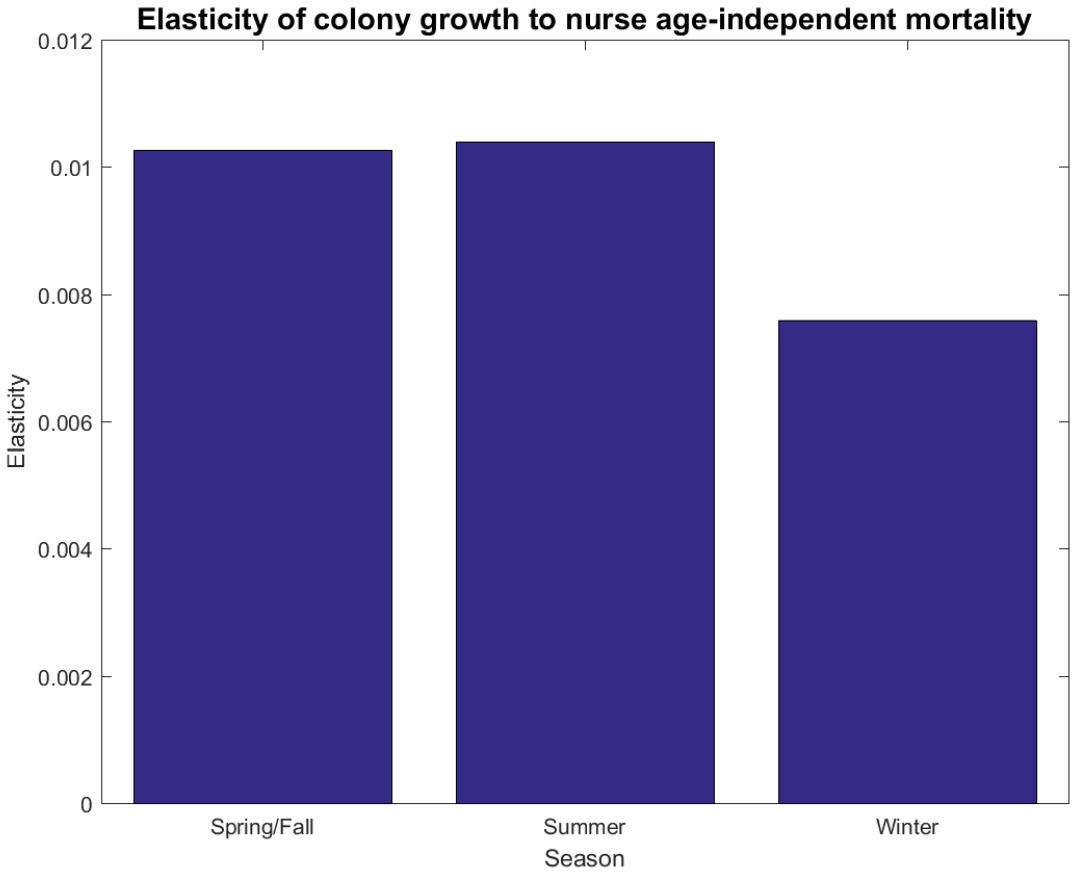


Figure 2

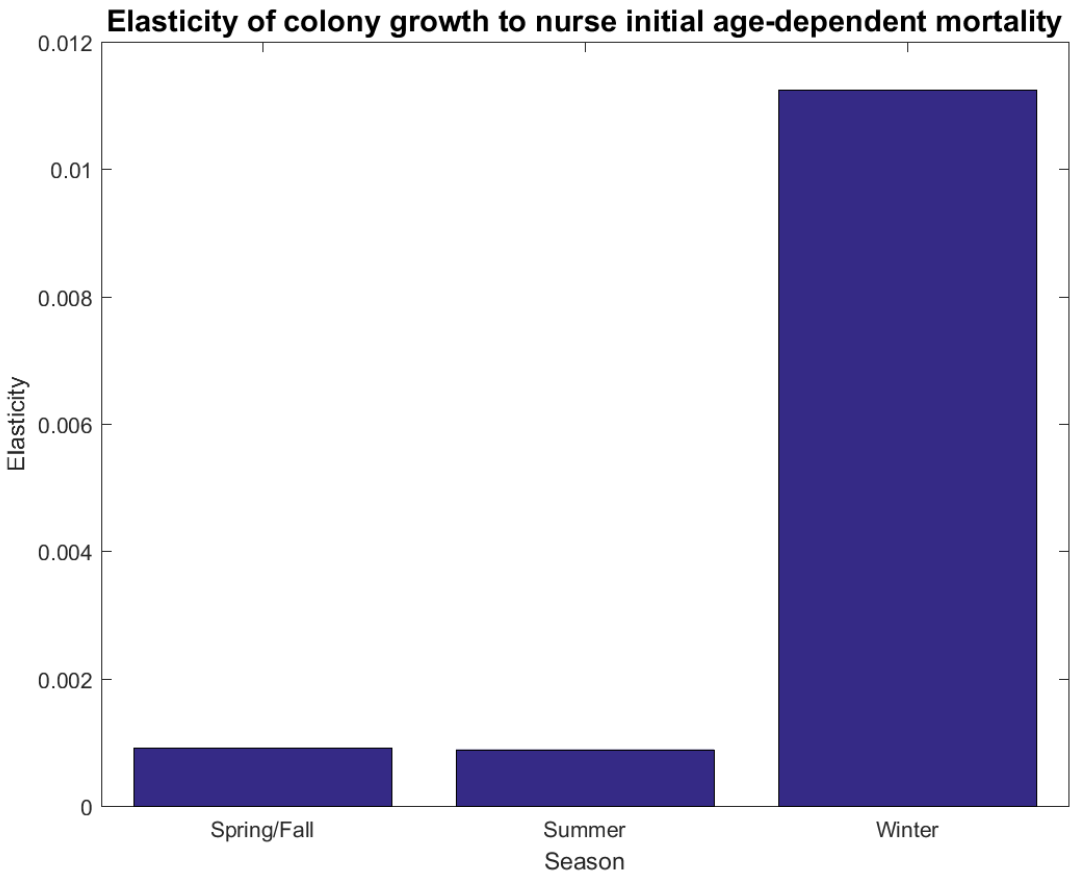

Elasticity of colony growth to nurse initial age-dependent mortality
0.012
0.01
0.008
0.006
0.004
0.002
0
Elasticity
Spring/Fall
Summer
Winter
Season

Figure 3

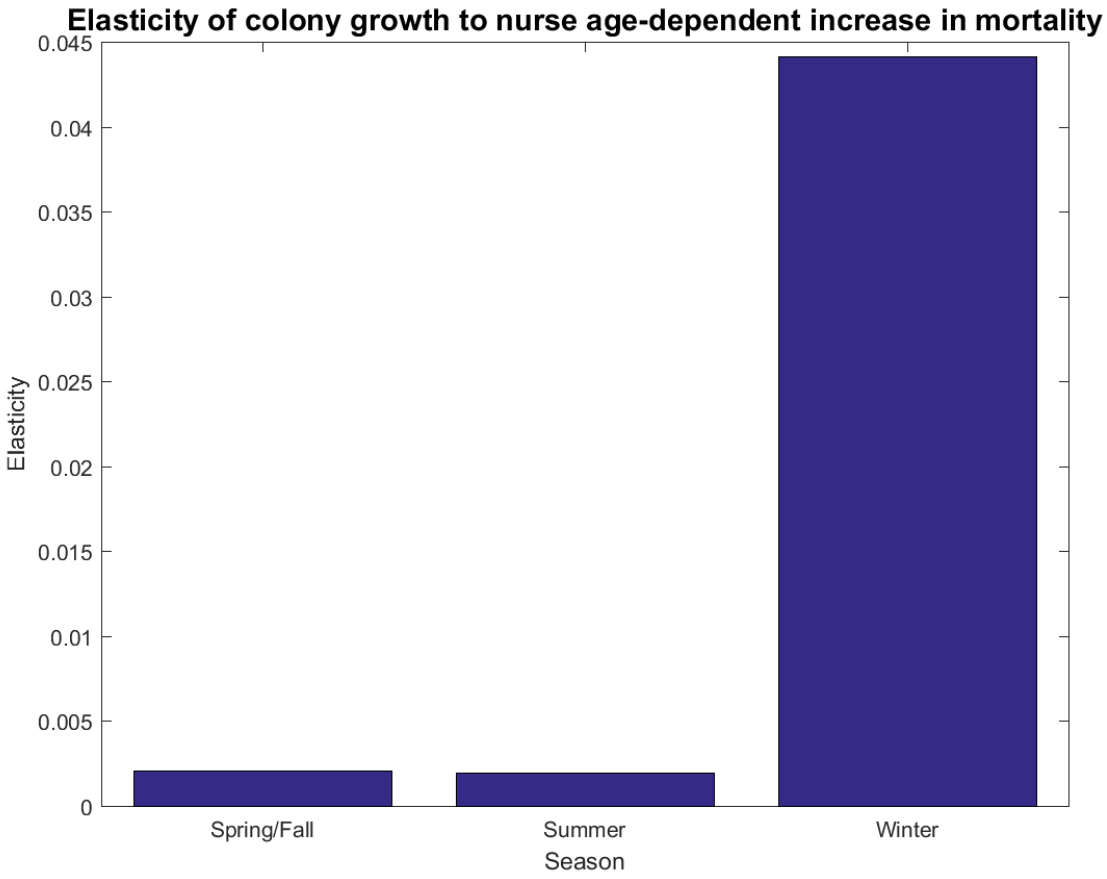

Elasticity of colony growth to nurse age-dependent increase in mortality
Elasticity
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
Spring/Fall
Summer
Winter
Season

Figure 4

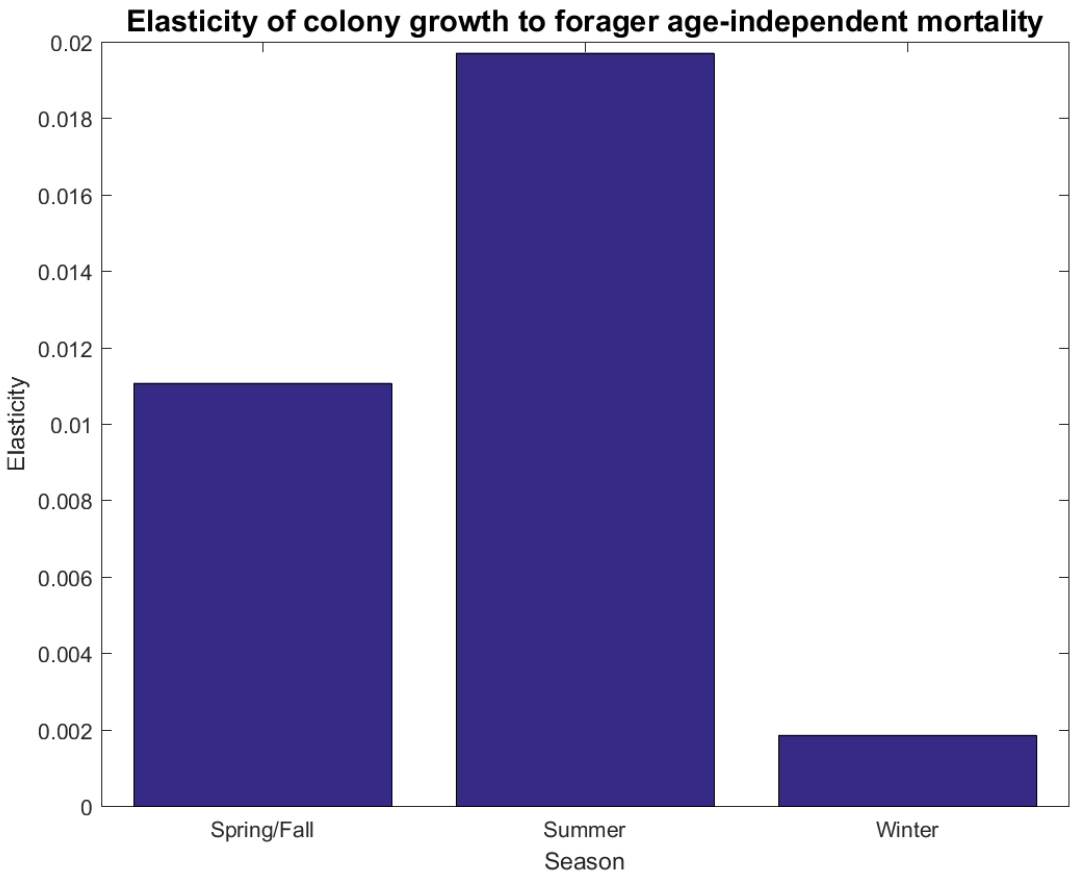

Elasticity of colony growth to forager age-independent mortality
0.02
0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
Elasticity
Spring/Fall
Summer
Winter
Season

Figure 5

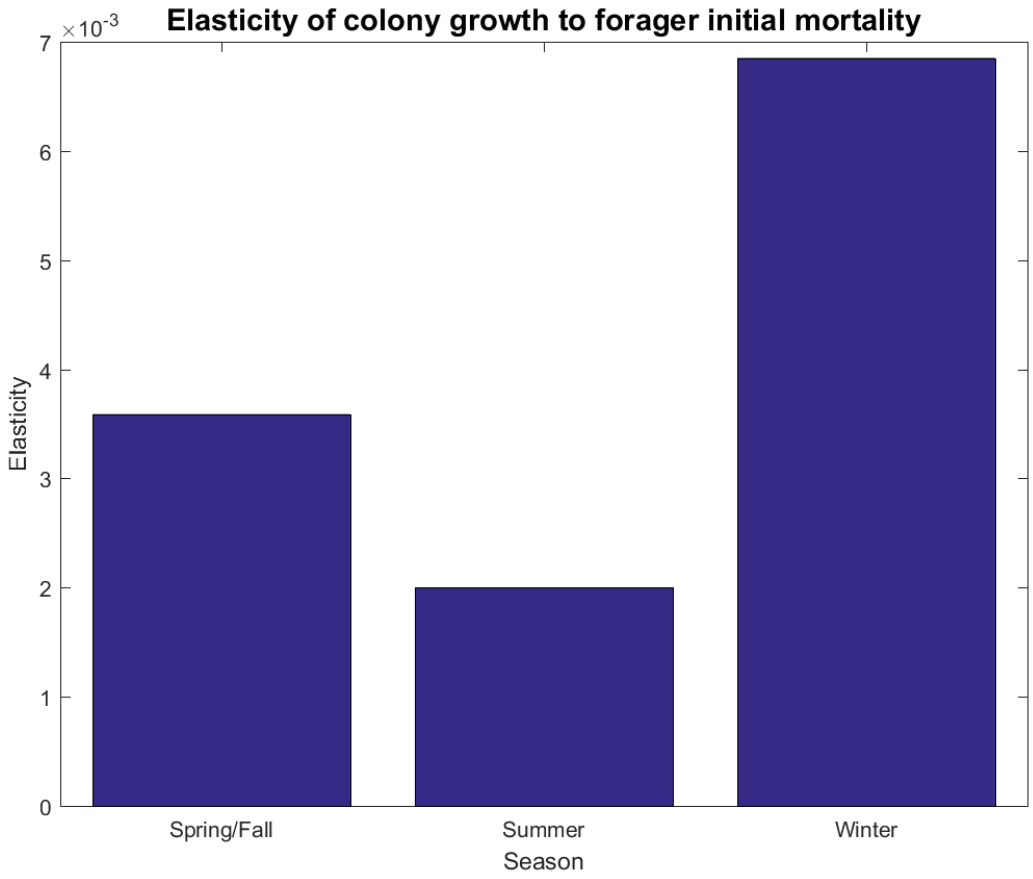
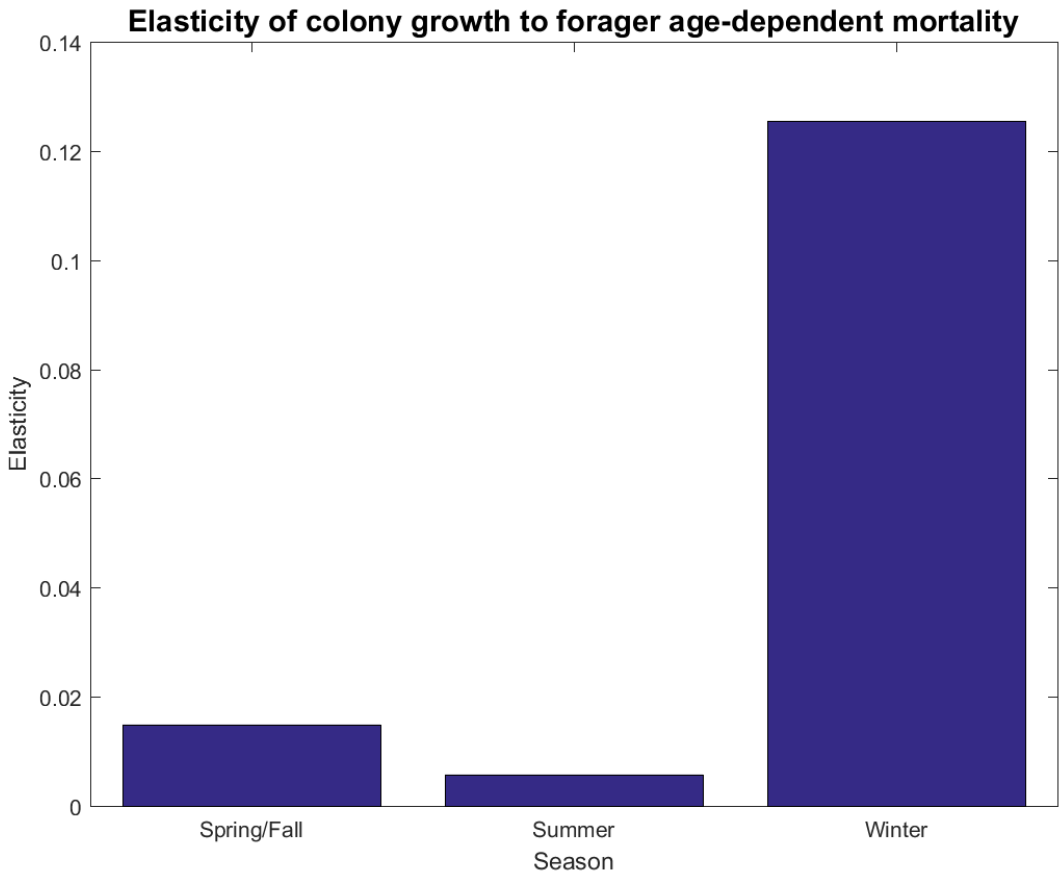

Elasticity of colony growth to forager initial mortality
×10^-3
7
6
5
4
3
2
1
0
Elasticity
Spring/Fall
Summer
Winter
Season

Figure 6


Elasticity of colony growth to forager age-dependent mortality
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Elasticity
Spring/Fall
Summer
Winter
Season

How Life History Shapes Optimal Patterns of Senescence: Implications from Individuals to Societies

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Abstract

The question of why organisms age has puzzled biologists for decades. The prevailing explanation is that senescence occurs because the force of selection declines with age. The amount of energy organisms invest in somatic maintenance is influenced by the probability of extrinsic mortality and the effect of somatic investment on survival. Senescence theory can also be used to explain how colonies decide how much to invest in the longevity of colony-members. Tests of the theory have focused on the role of extrinsic mortality, with fewer theoretical considerations of other factors affecting this trade-off, such as changes in the costliness of somatic maintenance or in the effect of individual lifespan on fitness of the group. We develop a decision theory model to evaluate how changing the marginal costs and benefits of longevity, along with extrinsic mortality, influence optimal worker lifespan in a social insect colony. We apply this

model to predict worker lifespan in honeybees. Our model predicts that higher extrinsic mortality favors shorter lifespan. However, increased lifespan is favored when marginal benefits are an increasing function of longevity. In honeybees, this explains how greater somatic investment is favored during gaps in brood rearing despite high mortality. We believe our approach expands the evolutionary theory of aging and can make quantitative predictions about the selective pressures shaping senescence in social systems.

Introduction

The phenomenon of senescence, the gradual deterioration in physiological function with age, has puzzled evolutionary biologists since it seems clearly detrimental to fitness, yet is a ubiquitous feature throughout the tree of life. Furthermore, organisms differ greatly in both longevity and the pattern of senescence (Piraino et al. 1996; Miller 2001; Morbey et al. 2005). Understanding the evolutionary pressures that shape differences in longevity is one of the major challenges of life history theory.

Evolutionary theory suggest that senescence occurs because of a decline in the force of selection with age (Medawar 1952; Williams 1957; Hamilton 1966). This decline in selection leads to a physiological decline in function with age because of an accumulation of late-acting deleterious mutations not eliminated by selection (Medawar 1952) or because of a trade-off between early life fecundity and later survival (Williams 1957; Nesse 1988). Disposable soma theory suggests a physiological mechanism for that trade-off; organisms have limited resources to allocate, so investing more energy in somatic maintenance means there is less available for reproduction (Kirkwood 1977). Selection should favor the optimal allocation of resources that maximizes fitness.

While evolutionary senescence theory was devised to explain senescence of individuals, this theory can also be used to understand the senescence of parts of a multicellular organism (e.g. Gardner and Mangel 1997) or of individuals in a functionally integrated superorganism, such as that of eusocial insect colonies (Seeley 1997; Lee 2003). Rather than maximizing individual reproductive value (Kozłowski 1993), selection in a superorganism acts on individual phenotypes to maximize their contribution to the

fitness of the colony. For social insects, for instance, where workers have little to no direct reproduction, worker phenotypes should evolve to maximize their contribution to the colony's survival, growth, and reproduction (Lee 2003).

One original prediction of evolutionary senescence theory was that a higher probability of extrinsic mortality should cause the evolution of faster senescence because the force of selection decreases more rapidly as the probability of surviving to older ages decreases (Medawar 1952; Williams 1957; Hamilton 1966). For a superorganism, higher extrinsic mortality should select for lower somatic investment and shorter lifespan of individuals. Extrinsic mortality can be defined as age-independent mortality and is usually thought of as mortality due to accidents or predation (e.g. Dowling 2012). Another prediction is that there should be a trade-off between reproductive effort and longevity (Kirkwood and Austad 2000).

There has been great interest in empirically testing these predictions. Experimental evolution studies have generally supported the prediction that higher extrinsic mortality leads to more rapid senescence (Gasser et al. 2000; Stearns et al. 2000) as well as the prediction of a trade-off between longevity and early fecundity (Rose 1984; Chippindale et al. 1993; Partridge et al. 1999; but see Partridge and Fowler 1992). In contrast, studies in wild populations have found mixed support for these predictions (Austad 1993; Holmes and Austad 1994; Keller and Genoud 1997; Bérubé et al. 1999; Wilkinson and South 2002; Reznick et al. 2004; Morbey et al. 2005; Ricklefs 2010; Healy et al. 2014).

There has been a great deal of work on refining the theoretical predictions of evolutionary senescence theory to help explain these conflicting findings. Contrary to Williams' original prediction, extrinsic mortality has no effect on senescence if a population is density-independent or if density affects survival of all age classes equally (Abrams 1993; Caswell 2007). However, it does select for faster senescence if density dependence acts uniformly on fertility (Abrams 1993). In addition, empirical tests often use predation as a source of extrinsic mortality (e.g. Reznick et al. 2004). However, while theory assumes extrinsic mortality is age-independent (Medawar 1952), predation is usually not random with respect to condition (Dowling 2012) and higher predation risk may therefore select for decreased, not increased, senescence (Chen and Maklakov 2012).

Furthermore, while previous models often assumed a linear effect of energy invested in reproduction on fertility or of energy invested in repair on somatic damage accumulation (Kirkwood and Rose 1991; Abrams and Ludwig 1995), there has been increasing recognition that the effect of energy investment in repair on mortality may be non-linear (Cichoń 1997; Cichoń and Kozłowski 2000; Mangel and Munch 2005; McNamara and Buchanan 2005; Munch and Mangel 2006). For instance, Cichoń (Cichoń 1997) found that a higher efficiency of repair selects for a longer lifespan.

Just as energy invested in somatic repair may have a non-linear effect on lifespan, in organisms or colonies that experience the senescence of parts, the lifespan of individual parts may have a non-linear effect on the fitness of the whole organism or colony. The effect of extrinsic mortality on worker lifespan has been examined in social

insects (Kramer and Schaible 2013). However, few theoretical studies have examined both non-linear effects of somatic repair on individual lifespan and non-linear effects of individual lifespan on fitness in social systems. Building on previous work, we seek to examine how the shapes of these relationships can influence the evolution of longevity in a social context.

We here present a decision theory model examining optimal worker lifespan in a social insect colony based on (a) the risk of extrinsic mortality, (b) the effect of worker lifespan on colony productivity, defined as the colony's rate of acquisition of energetic resources (marginal benefits of lifespan) and (c) the effect of somatic investment in workers on worker lifespan (marginal costs of lifespan). Decision theory is a mathematical framework dealing with optimal decision making under risk or uncertainty (Peterson 2009) that has been widely applied in ecology (McNamara and Houston 1980; McNamara and Buchanan 2005; Nesse 2005; Bateson 2007). Decision theory is a logical framework for modeling social individuals as investments by a colony, with some risk (extrinsic mortality) and expected value; it can easily incorporate nonlinearity in the costs and benefits of the investment to determine the optimal investment level. This framework can also incorporate empirical data and predict how multiple factors interact to determine a colony's optimal investment in the lifespan of its colony members.

We examine this question using honeybees (*Apis mellifera*) as an excellent model system for expanding on evolutionary aging theory. Honeybee workers have extraordinary phenotypic plasticity in lifespan, influenced by season and behavioral role (Remolina et al. 2007; Münch and Amdam 2010). As predicted by disposable soma

theory, the transition from in-hive work to foraging, with an accompanying increase in extrinsic mortality, also results in faster physiological senescence and a shorter lifespan (Rueppell et al. 2007; Dukas 2008). Worker senescence is under individual and social control (Amdam et al. 2005), making it an emergent property of the colony. Greater protein consumption results in greater longevity, meaning potentially long lived workers are more costly to produce (Amdam et al. 2004; Münch and Amdam 2010). Conveniently, measuring resource allocation among individual workers is much easier than measuring allocation among functional systems of an organism. Honeybee colonies are highly integrated units; although there is the potential for conflict, in the matter of colony survival the interests of workers are largely aligned (Seeley 1997).

Methods

We model worker bees as an investment by the colony (the agent whose utility is being maximized). Selection should act on colonies to invest in somatic maintenance of workers in a way that optimizes the net contribution of its workforce to colony productivity, a reasonable proxy for colony fitness in honeybees because it determines how much energy can be allocated among the parent and all offspring colonies. We define the intrinsic lifespan as the average lifespan of a worker not killed by any extrinsic source of mortality. Intrinsic lifespan, n, is the variable being optimized in the model.

We assume the colony can invest energetic resources to increase the durability of workers (more durable workers have a longer intrinsic lifespan). In honeybees, increasing the size of a worker's protein reserve results in an increased lifespan, but imposes an increased cost because the worker must consume more pollen (Crailsheim

et al. 1992; Amdam and Omholt 2002; Amdam et al. 2004; Alaux et al. 2010). Investing sufficient resources to produce workers with an intrinsic lifespan of *n* days imposes a cost, which we call C_n. We then define the marginal cost, $\hat{C}_n$, as the increase in an average worker's resource consumption resulting from increasing its intrinsic lifespan from *n-1* to *n* days. In principle, we can empirically estimate the shape of the cost curve by measuring the impact of protein consumption on intrinsic lifespan. We can define total cost of workers with intrinsic lifespan *n*:

$$C_n = \sum_1^n \hat{C}_n$$

We next define the marginal benefit, $\hat{B}_n$, as the increase in colony productivity resulting from an average worker's lifespan increasing from *n-1* to *n* days. In addition, workers have a daily probability of age-independent extrinsic mortality, *m*. The colony must pay an upfront cost, C_n, to produce workers of sufficient durability to have an intrinsic lifespan *n* days, regardless of whether they are killed by extrinsic mortality before reaching age *n*, but marginal benefits $\hat{B}_n$ are only realized once workers survive to age *n*. We can therefore define the expected payoff of workers with an intrinsic lifespan of *n* days as:

$$P_n = \sum_1^n (\hat{B}_n (1-m)^n) - \sum_1^n \hat{C}_n$$

We then manipulate the shapes of the cost and benefit functions to examine how their shapes affect the colony's optimal intrinsic worker lifespan. We model cases in which the benefit function is linear (marginal benefits are constant), exponential (marginal benefits are monotonically increasing), saturating (marginal benefits are

monotonically decreasing), or sigmoidal (marginal benefits increase up to some point and then decrease) with respect to intrinsic lifespan. We model the marginal benefits as a simple recursive function; this form was chosen arbitrarily as a convenient function that could take on various shapes by altering two parameters (see Cichoń 1997). When parameter $b_1 > 1$, the marginal benefits are decreasing, when $b_1 < 1$, the marginal benefits are increasing, and when $b_1 = 1$, the marginal benefits are constant with respect to n. When parameter $b_2 = 0$, $\hat{B}_n$ increases or decreases monotonically; when $b_2 > 0$, $\hat{B}_n$ increases and then decreases (B_n is sigmoidal). We define the marginal benefits function:

$$\hat{B}_n = \frac{\hat{B}_{n-1}}{b_1}(1 - b_2(n-1))$$

Similarly, we model cases in which the cost function is linear (marginal costs are constant), exponential (marginal costs are increasing), and saturating (marginal costs are decreasing). When parameter $c_1 > 1$, the marginal costs are decreasing, when $c_1 < 1$, the marginal costs are increasing, and when $c_1 = 1$, the marginal costs are constant with respect to n. We define the marginal cost function:

$$\hat{C}_n = \frac{\hat{C}_{n-1}}{c_1}$$

The exact values of C_n and B_n do not affect our general result but their ratio may influence the optimal intrinsic lifespan. We define parameter q as the ratio of the initial value of $\hat{B}_n$ to the initial value of $\hat{C}_n$ and we examine various values of parameter q. We define $\hat{B}_1$ as the initial value of $\hat{B}_n$ and $\hat{C}_1$ as the initial value of $\hat{C}_n$. We assign $\hat{C}_1$ a value of 1 and we define $\hat{B}_1$ as a function of $\hat{C}_1$ and q:

$$\hat{B}_1 = q\hat{C}_1$$

Finally, we define the optimal intrinsic lifespan, *I,* as the point *n* where P_n is maximized:

$$I = \text{argmax}(P_n)$$

Results

Our model shows that when the costs and benefits of worker lifespan are linear with respect to lifespan, a decrease in extrinsic hazards increases the optimal intrinsic lifespan (Figure 1). This finding is consistent with existing senescence theory, which predicts that, all else being equal, longer lifespan should evolve when risks of accidental mortality are low (Kirkwood and Austad 2000).

We also find that increasing *q*, the ratio of initial marginal benefits to initial marginal costs, increases the optimal intrinsic lifespan (Figure 2). This means that increased worker lifespan should occur if the same degree of somatic maintenance can be achieved at a lower cost (higher efficiency of repair) or when protein is cheaper (when pollen is more abundant). We also expect increased worker lifespan to be optimal if the contribution per worker to colony productivity increases over its whole lifespan.

Extending beyond the predictions of previous theory, our model shows that a marginal costs function that increases with respect to lifespan favors a shorter intrinsic lifespan compared to when marginal costs are constant or decreasing (Figure 3).

Increasing costs might occur when physical wear is multiplicative rather than cumulative (Cichoń 1997) or when one kind of wear increases other maintenance costs.

In addition, we find a marginal benefits function that increases with respect to lifespan favors longer intrinsic lifespan, while a decreasing marginal benefits function favor shorter intrinsic lifespan (Figure 4). Increasing marginal benefits occur when an individual's value increases with age. For a social insect worker, it may, for example, reflect an increase in an individual's contribution to the colony with age because of learning. For a solitary organism, it could reflect an increase in fecundity with age.

Discussion

Major evolutionary theories of aging agree that the fundamental cause of senescence is a decline in the force of selection with age. The disposable soma theory of aging explains senescence as a decline in physiological function caused by wear and tear that is allowed to accumulate rather than being repaired (Kirkwood and Rose 1991); the rate of senescence, and therefore lifespan, is a function of the amount of resources allocated to somatic repair rather than growth or reproduction (Kirkwood and Austad 2000). Natural selection acts on this allocation to maximize fitness. In an organism or colony that experiences senescence of parts, selection acts on the level of somatic investment in individual parts to maximize the fitness of the whole organism or colony.

Much current debate in evolutionary aging research centers on the role of extrinsic mortality in shaping the evolution of lifespan. Early evolutionary theories of aging predicted that greater extrinsic mortality selects for less allocation to maintenance and faster senescence (Medawar 1952; Hamilton 1966; Kirkwood 1977), while

subsequent work suggests the relationship may be more complex (Law 1979; Abrams 1993; Baudisch 2011; Chen and Maklakov 2012).

There has also been increasing recognition that nonlinearity in the effects of somatic investment may be important to the evolution of lifespan (Cichoń 1997; Munch and Mangel 2006). Our model extends previous work by explicitly considering the effect of nonlinearities in both the energetic cost and the fitness benefits of changes in the intrinsic lifespan of individuals in a social system.

We've chosen to focus on honeybees as a model system since they have a phenotypically plastic worker lifespan that is influenced by resource investment. In addition, the effects of resource investment on worker lifespan and the effects of worker lifespan on colony fitness are both empirically measurable, allowing us to estimate the shapes of benefit and cost curves and make testable predictions. We've tailored this model to an example system to demonstrate how this modeling approach can make testable predictions about how ecological circumstances affect the evolution of lifespan.

Our model results agree with one of the main predictions of existing theory: that, all else being equal, higher extrinsic mortality should select for shorter intrinsic lifespan (Figure 1). This means that we expect colonies to invest fewer resources in workers that have a greater risk of death from external hazards such as predation. In honeybees, the largest change in extrinsic mortality occurs at the transition from in-hive work to foraging (Dukas 2008). As predicted, workers do experience reduced protein investment and consequently faster physiological senescence at the behavioral

transition to foraging (Münch and Amdam 2010). Our model predictions are therefore consistent with the pattern of senescence associated with age polyethism in honeybees.

Our model also suggests that colonies should invest more in worker somatic maintenance when the benefit to cost ratio increases, e.g. when increased lifespan can be achieved at a lower cost (Figure 2). For honeybees, protein investment in workers should increase when pollen is more abundant. This prediction could be empirically tested in honeybees by experimentally manipulating the amount or quality of protein available to entire colonies and measuring any changes in the intrinsic lifespan of adult workers. For other organisms, the return on investment in maintenance can change depending on the ecological circumstances. For instance, high resource abundance may favor phenotypes good at acquiring resources, allowing increased investment in reproduction without decreased investment in maintenance (Reznick et al. 2000).

Our model shows that an increasing marginal costs function selects for decreased intrinsic lifespan, compared to constant or decreasing marginal costs (Figure 3). Increasing marginal costs are likely to be a widespread pattern in nature because of the “low hanging fruit” principle; if there are multiple physiological mechanisms that can increase lifespan, organisms should first invest in pathways with the lowest unit cost. Increasing marginal costs can also occur when one kind of somatic damage makes other forms of maintenance costlier. For instance, wing wear, a major component of senescence in bees (Foster and Cartar 2011), probably increases the metabolic cost of flight; as a result workers with greater accumulated wing wear would require more

energy to feed and also experience greater oxidative damage, which, if unrepaired, further accelerates senescence (Sohal and Weindruch 1996).

Our results also show that a pattern of increasing marginal benefits selects for greater intrinsic lifespan (Figure 4). If the marginal benefits function is constant, colonies should be indifferent between short-lived workers and long-lived workers as long as the total number of worker-days stays the same. However, if the benefits of a worker are non-linear with respect to age, colonies should prefer long-lived to short-lived workers when the benefits are increasing, but prefer short-lived to long-lived workers when the benefits are decreasing. In honeybees, this suggests that workers should live longer when older bees are more valuable than younger bees; this situation occurs when learning increases an individual's value to the colony. For instance, in honeybees, older, more experienced foragers usually act as scouts during the house hunting process, making them especially valuable to the swarm during that period (Gilley 1998). Therefore, this model predicts long-lived workers to be more valuable at that time in the colony life cycle.

A pattern of increasing marginal benefits may also occur when there is a threshold effect or a minimum worker longevity that needs to be reached for a colony to survive a period where no new workers are produced. In honeybees, one such broodless period is winter, when workers are known to experience their slowest rate of aging (Amdam and Omholt 2002); another broodless period occurs immediately following reproductive swarming (Winston 1987). Our model predicts that changing the shape of the marginal benefit function alone can increase the optimal intrinsic worker

lifespan even when extrinsic mortality is high, leading to the unusual prediction that honeybee workers may age slower rather than faster in colonies that have recently swarmed (Figure 5). This prediction could be tested empirically by comparing the protein status of workers from recently swarmed colonies to those from similarly sized colonies that have not swarmed; protein status could be measured as level of stored vitellogenin, a lipoprotein that increases immune function and longevity in honeybees (Amdam et al. 2004; Seehuus et al. 2006) and which requires protein consumption to produce (Münch and Amdam 2010). Colonies should invest more in worker maintenance during swarming despite high losses due to extrinsic mortality because the benefit of long lived workers exceeds the opportunity cost of additional lower value short lived workers.

This work extends evolutionary senescence theory by providing a framework for examining how nonlinear costs and benefits affect the optimal lifespan in a social system. This model framework may be used with empirically estimated benefit and cost functions to make specific, testable predictions about how lifespan changes under different circumstances in organisms like the honeybee with adaptive plasticity as well as how lifespan evolves in different populations experiencing different ecological circumstances.

Tables

Table 1. List of model variables.

Variable	Definition
n	Intrinsic worker lifespan, defined as the average lifespan of a worker not killed by any extrinsic (age-independent) source of mortality
P_n	Colony productivity resulting from an intrinsic worker lifespan of *n* days
B_n	Contribution to the colony of workers with intrinsic lifespan of *n* days
$\hat{B}_n$	Marginal benefit from increasing worker intrinsic lifespan from *n*-1 to *n* days
C_n	Cost to the colony of producing workers with intrinsic lifespan *n*
$\hat{C}_n$	Marginal cost of increasing worker intrinsic lifespan from *n*-1 to *n* days
I	Optimal worker intrinsic lifespan

Table 2. List of model parameters.

Parameter	Definition	Value
m	Daily probability of extrinsic worker mortality	0 – 0.98
b_1	Parameter governing shape of benefits function (exponential, linear, or saturating)	0.7 – 1.3
b_2	Parameter governing shape of benefits function (sigmoidal or non-sigmoidal)	0 – 0.6
c_1	Parameter governing shape of costs function (exponential, linear, or saturating)	0.7 – 1.3
$\hat{B}_1$	Initial marginal benefit of worker with intrinsic lifespan of 1	1 – 50
$\hat{C}_1$	Initial marginal costs of worker with intrinsic lifespan of 1	1
q	Ratio of initial marginal benefits ($\hat{B}_1$) to initial marginal costs ($\hat{C}_1$)	1 – 50

Figure Legends

Figure 1: Effect of extrinsic mortality on optimal worker intrinsic lifespan. As daily probability of extrinsic mortality (parameter *m*) increases, the colony's optimal worker intrinsic lifespan decreases. We explored 50 values of *m* from 0 to .98, with other parameters held constant. Parameter $q = 6$, $b_1 = 1$, $b_2 = 0$, $c_1 = .95$.

Figure 2: Effect of benefit to cost ratio on optimal worker intrinsic lifespan. Increasing parameter *q*, the ratio of the initial marginal benefits to initial marginal costs, increases the colony's optimal worker intrinsic lifespan. We explored 50 values of *q* from 1 to 50 with other parameters held constant. Parameter $m = .1$, $b_1 = 1.1$, $b_2 = 0$, $c_1 = .9$.

Figure 3: Effect of cost function on optimal worker intrinsic lifespan. Parameter c_1 governs the shape of the marginal cost function; $c_1 = 1$ implies that the marginal cost is constant (costs increase linearly with worker lifespan), $c_1 > 1$ implies that marginal costs are decreasing (costs increase in a saturating way with lifespan), and $c_1 < 1$ implies that marginal costs are increasing (costs increase exponentially with lifespan). We find that the optimal worker lifespan decreases when marginal costs are increasing (i.e. when additional energetic investments produce smaller and smaller increases in worker lifespan) and increases sharply when marginal costs are decreasing. We examined 41 values of parameter c_1, from .7 to 1.3. Other parameters held constant at $m = .1$, $q = 6$, $b_1 = 1.1$, $b_2 = 0$.

Figure 4: Effect of benefits function on optimal worker intrinsic lifespan. Parameters b_1 and b_2 govern the shape of the marginal benefit function. Parameter $b_1 = 1$ means the

marginal benefit is constant (benefits increase linearly with lifespan), $b_1 > 1$ means marginal benefits are decreasing (benefits function is saturating), and $b_1 < 1$ implies that marginal benefits are increasing (benefits function is exponential). Parameter b_2 governs whether the benefits function is sigmoidal; when $b_2 = 0$, the marginal benefits are monotonically increasing or decreasing, and when $b_2 > 0$, the marginal benefits increase to a certain point and then decrease (benefits function is sigmoidal). The optimal worker lifespan increases when marginal benefits are increasing ($b_1 < 1$) and decreases when marginal benefits are decreasing ($b_1 > 1$). We examined 51 values of parameter b_1, from .7 to 1.3 and 51 values of parameter b_2 from 0 to .6. Other parameters held constant at $m = .11$, $q = 4$, $c_1 = .95$.

Figure 5. Effect of benefits function on optimal worker lifespan in honeybees. As an application of this framework, we model the broodless period after swarming in honeybees as a change in the shape of the marginal benefits function. a) Left panel show optimal worker lifespan when the benefit function is linear. Parameter $b_1 = 1$. b) During swarming, there is a threshold worker lifespan below which colony survival is close to zero. The marginal benefit of each worker increases sharply above this threshold (benefits increase exponentially rather than linearly). An exponential benefits function results in increased optimal worker lifespan. Parameter $b_1 = .95$. Other parameters held constant at $q = 5$, $c_1 = 1$, $m = .1$.

Figures

Figure 1

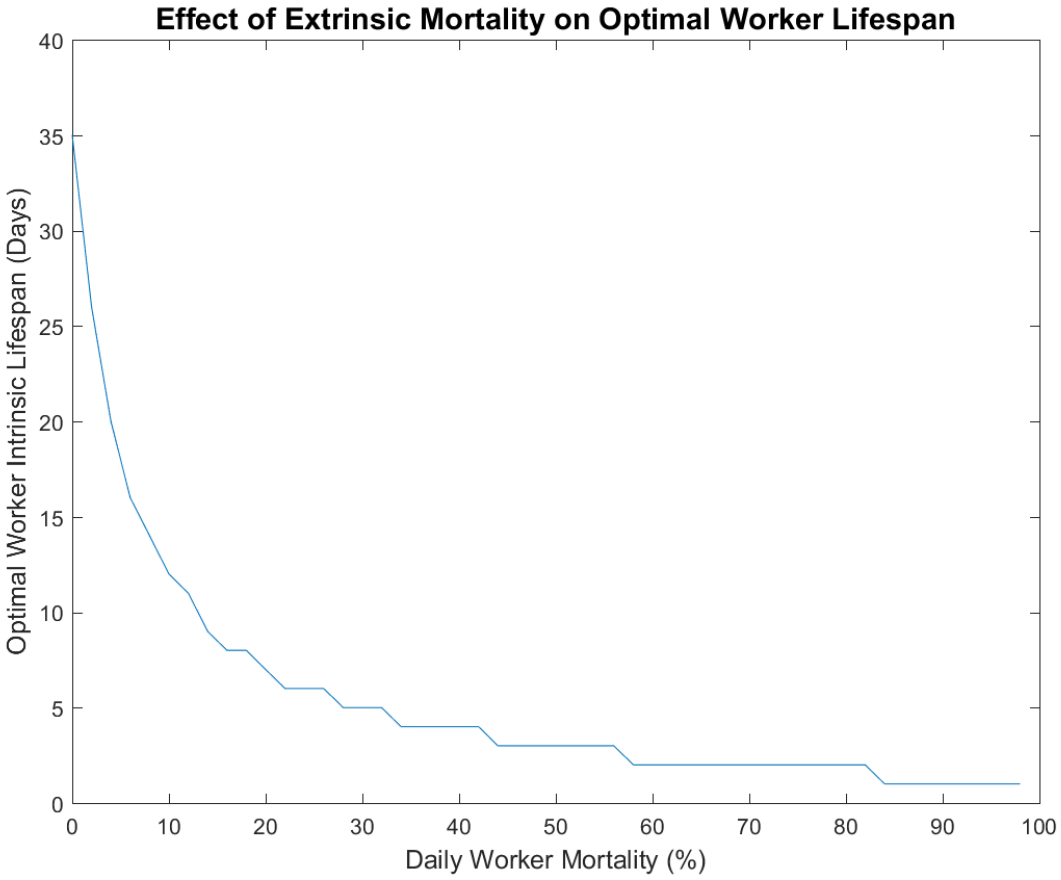


Figure 2

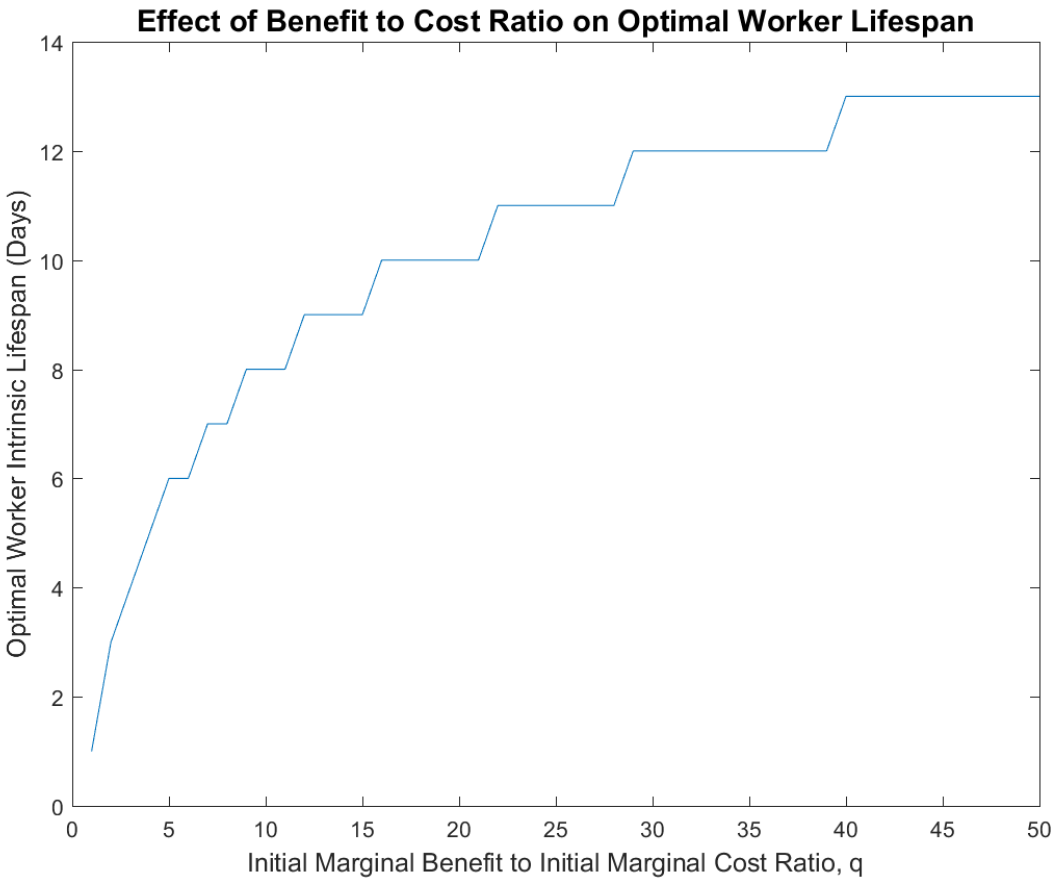

Effect of Benefit to Cost Ratio on Optimal Worker Lifespan
Optimal Worker Intrinsic Lifespan (Days)
Initial Marginal Benefit to Initial Marginal Cost Ratio, q
0
2
4
6
8
10
12
14
0
5
10
15
20
25
30
35
40
45
50

Figure 3

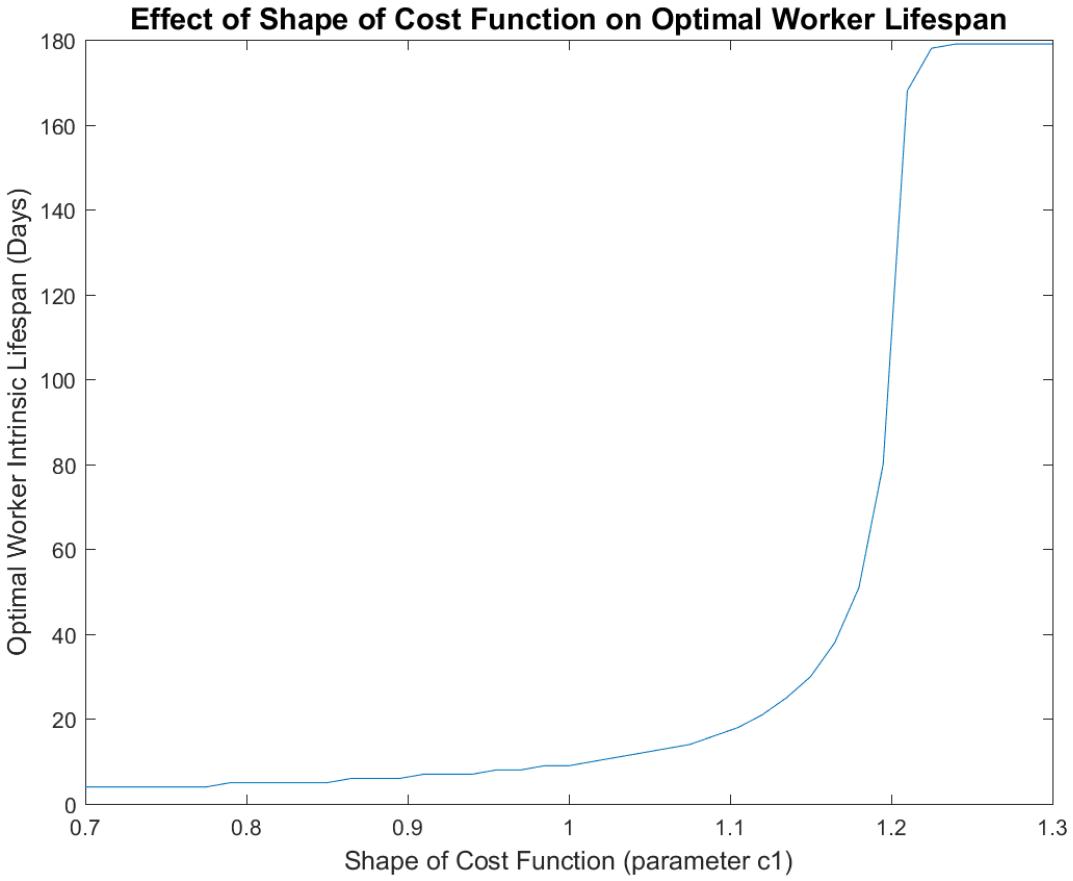

Effect of Shape of Cost Function on Optimal Worker Lifespan
Optimal Worker Intrinsic Lifespan (Days)
Shape of Cost Function (parameter c1)
0
20
40
60
80
100
120
140
160
180
0.7
0.8
0.9
1
1.1
1.2
1.3

Figure 4

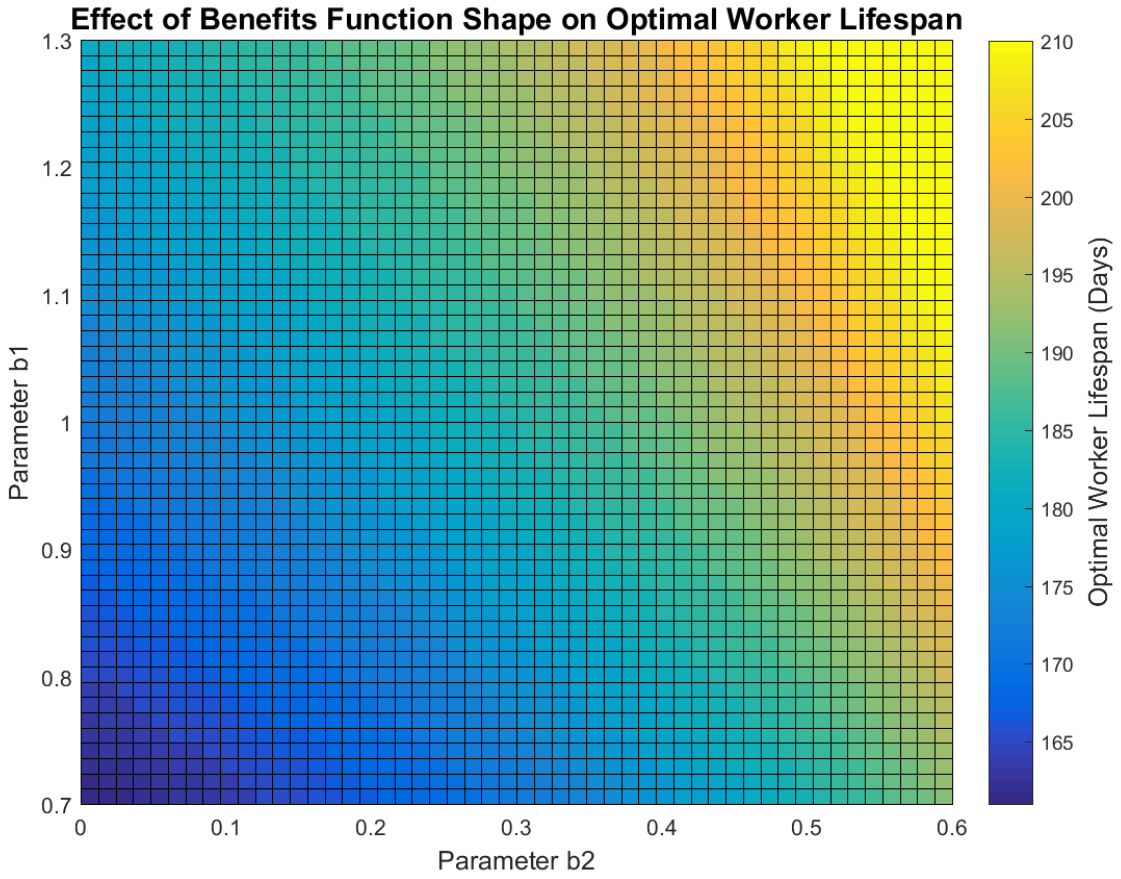
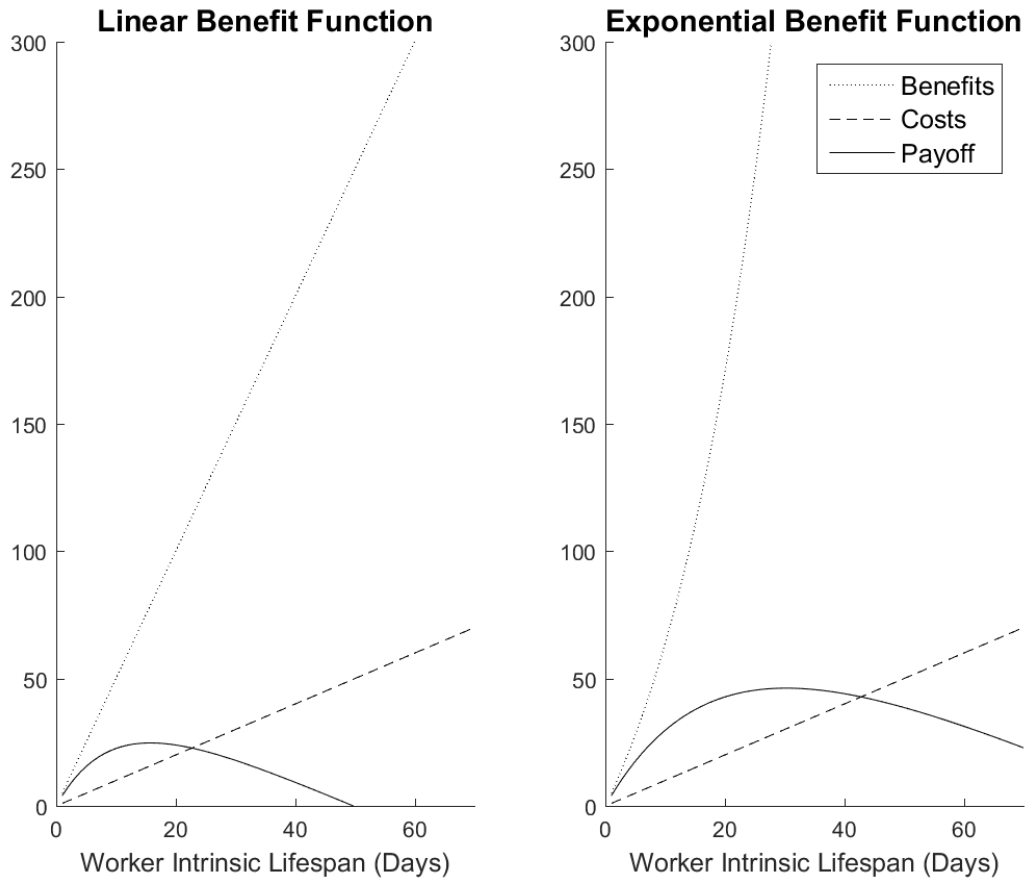

Effect of Benefits Function Shape on Optimal Worker Lifespan
Parameter b1
Parameter b2
Optimal Worker Lifespan (Days)
1.3
1.2
1.1
1
0.9
0.8
0.7
0
0.1
0.2
0.3
0.4
0.5
0.6
210
205
200
195
190
185
180
175
170
165

Figure 5


Linear Benefit Function
Exponential Benefit Function
Benefits
Costs
Payoff
300
250
200
150
100
50
0
0
20
40
60
Worker Intrinsic Lifespan (Days)
Worker Intrinsic Lifespan (Days)

Coordination Between the Sexes Constrains the Optimization of Reproductive Timing in Honey Bee Colonies

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Abstract

Honeybees are an excellent model system for examining how trade-offs shape reproductive timing in organisms with seasonal environments. Honeybee colonies reproduce two ways: producing swarms comprising a queen and thousands of workers or producing males (drones). There is an energetic trade-off between producing workers, which contribute to colony growth, and drones, which contribute only to reproduction. The timing of drone production therefore determines both the drones' likelihood of mating and when colonies reach sufficient size to swarm. Using a linear programming model, we ask when a colony should produce drones and swarms to maximize reproductive success. We find the optimal behavior for each colony is to produce all drones prior to swarming, an impossible solution on a population scale because queens and drones would never co-occur. Reproductive timing is therefore not

solely determined by energetic trade-offs but by the game theoretic problem of coordinating the production of reproductives among colonies.

Introduction

All organisms must make trade-offs in how they allocate limited resources among growth, maintenance, and reproduction. The seasonal availability of many critical resources, such as food or habitat, further complicate these trade-offs. In addition, the total energy budget is not static through time; allocating more energy to growth at one point in time means more total energy available in the future, but at the expense of immediate investment in reproduction.

Eusocial insects, such as the European honey bee (*Apis mellifera*) are a good model system for studying these trade-offs. Honey bees live in colonies composed of a single reproductive female and thousands of functionally sterile workers (Winston 1987). The colony has a shared energy budget and mostly shared reproductive interests (Seeley 1997; Tarpy et al. 2004). Honey bee societies comprise three morphological castes: workers, queens, and drones. Investment in each of these castes can conveniently represent the different methods of allocating limited resources toward different life history requirements. Workers contribute to colony growth and survival by performing all foraging, brood care, and defense (Winston 1987). The queen's sole job is to lay eggs; the colony rears new queens only when the colony is ready to reproduce or when the original queen requires replacement (Winston 1987; Tarpy et al. 2004). Drones are the male reproductives of a honey bee colony. The drones consume colony resources and perform no work; their sole purpose is to pass on the colony's genes by mating with queens from other colonies (Winston 1987). Thus, workers typically eject

drones from the colony in early fall once they have little chance of mating (Winston 1987).

Honey bees, along with army ants (Hölldobler et al. 1985; Franks and Hölldobler 1987), stingless bees (Santos-Filho et al. 2006), and some social wasps (Solis et al. 1998), form new colonies by swarming, a process in which a new queen is reared and the entire colony fissions, with a fraction of the worker population going with the old queen (called the prime swarm) and the rest remaining in the original colony with the new queen. While drones can be considered an investment in reproduction, workers are therefore an investment in both growth and reproduction. Previous theoretical work on the timing of reproductive investment in annual social insect colonies has found there is an optimal time to switch from pure growth to pure reproduction to maximize the number of reproductives produced, otherwise called a "bang-bang strategy" (Oster and Wilson 1978; Mitesser et al. 2007; Poitrineau et al. 2009). However, because the swarm is a large part of the colony's investment in queens, the annual reproductive success of a honeybee colony can more appropriately be considered as the total of all surviving colonies related to the parent colony: the original colony headed by a daughter queen, the prime swarm headed by the original queen, afterswarms headed by daughter queens, and any colonies fathered by the parent colony's drones.

In the swarm-founding social insects, there has been much work on optimal sex ratio (Bulmer 1983; Page and Metcalf 1984), swarm fraction (Macevicz 1979; Rangel and Seeley 2012), and queen choice (Tarpy et al. 2016), but very little on reproductive timing. The timing of investment in reproduction is extremely important to reproductive

success. Temperate honey bee colonies persist year-round, but their food supply is only present in spring and summer when flowers are in bloom (Seeley and Visscher 1985). Thus, colonies have a limited period each year in which to grow, reproduce, and store enough food to survive winter.

Because of the dynamics of exponential growth, investing directly in reproduction (via production of drones) rather than growth (via production of workers) early in the year, leads to a smaller final colony size than could be achieved by investment in growth first and reproduction later in the year. Because each worker produced contributes to the production of future workers (i.e. an investment with compounding interest), investing in drones early siphons resources away from that capital and thereby reduces the energy available to produce either drones or workers later. Since swarming is regulated at a whole-colony level, and colonies must attain sufficient size to swarm, the level of investment in drones versus workers determines the amount of energy the colony can later invest in both swarms and drones, as well as when swarming will occur. The timing of drone production is therefore important in driving both male and female components of reproductive success.

In addition to affecting the total energy budget, the timing of reproductive investment also affects drones' chances of mating. If mated successfully, a drone can be expected to be the father of approximately 1/12th of the workers and daughter queens of a new colony (Baer 2005). However, because there are many times more drones than queens in the population, each individual drone is very unlikely to mate successfully (Baer 2005). A drone's reproductive success depends on the supply of queens with

which to mate, so drones should be supported only when other colonies are making swarms, regardless of what would be the best time to produce them based on a colony's energetic trade-offs between reproduction and somatic growth. Swarms are only successful when there are sufficient drones available to mate with the virgin queen; however, because there are many more drones than queens, almost all virgin queens in a population mate successfully. A new queen's reproductive success is mostly driven by how large her worker population is and how long the colony has to gather enough resources to survive winter.

We expect all colonies in a population to evolve to produce drones and swarms at the time that maximizes their own reproductive success. However, the best time for each colony to produce male and female reproductives will depend on when other colonies reproduce, as well as the energetic trade-off between growth and reproduction. If the optimal timing of drone production based on colony growth dynamics results in each colony producing drones and queens at the same time, we should expect all colonies to produce drones at the time that both maximizes the drones' mating success and maximizes colony growth. If the optimal time for a colony to produce drones differs from the optimal time for a colony to produce queens, there is a potential game theoretic conflict among colonies. Each colony would maximize its fitness by producing drones at the energetically optimal time if other colonies produce queens at that time, but all other colonies would also prefer to produce drones at that time as well. Since colonies need to produce drones when other colonies produce queens and vice versa, all colonies cannot produce drones at the energetically optimal

time because that would lead to the worst fitness option, failure to coordinate among colonies.

To fully understand the dynamics of this system, we present a mathematical model of honey bee swarm and drone production addressing two questions: a) when should a honey bee colony produce and eject drones to optimize its yearly reproductive success? and b) how does the energetic trade-off between growth and drone production interact with the drone mating success in shaping the timing of colony reproduction?

To answer our questions, we have constructed a linear programming model (Fourer et al. 2003) of the timing of swarming and drone production in a temperate honey bee colony. Linear programming is a mathematical tool for finding optimal solutions in a system with many interacting variables, which, to the best of our knowledge, has not been previously applied to the question of reproductive timing in insect societies. An advantage of this approach to the question of reproductive timing is that, unlike most previous work (Page and Metcalf 1984), it does not require us to determine what fraction of investment in workers should be counted as an investment in the swarm and what should be counted as an investment in drones. In our model, the objective function to be maximized is annual colony fitness and the constraints involve a limited energy budget allocated among workers, drones, and food stores. The outputs of the model we are most interested in are the optimal time for the colony to invest in daughter queens, via swarming, and the optimal times for the colony to invest in drones.

We also compared two alternative scenarios in the model. In Scenario 1, we calculate the optimal pattern of drone and worker production for a focal colony, assuming other colonies in the population do not change their reproductive behavior in response to the selection pressure we calculate. Instead we assume that queens are available for the focal colony to mate with on a fixed day, regardless of whether it is the optimal time to produce queens.

In Scenario 2, we calculate the optimal pattern of drone and worker production for a focal colony, assuming all colonies in the population produce queens on the optimal day. The purpose of this scenario is to examine how the need to coordinate reproductive timing with other colonies changes the optimal behavior. Realistically, we would not really expect all colonies to produce swarms and drones on the same day because of natural variation in the strength and growth rate of colonies; this variation may partly alleviate a potential conflict between colonies. However, our model ignores inter-colony variability to examine the strongest version of the potential conflict to better understand its effects on reproductive timing.

Results

Scenario 1

Under the assumption that drone mating success is independent of when swarms are produced by the focal colony, we find that, over a broad range of parameters (Supplementary Figs S2-S8), swarms and drones do not co-occur in the optimal solution (Fig 1). The globally optimal solution is for colonies to produce all drones early in

summer and eject all drones prior to swarming (Fig 1). In addition, the optimal swarm date is late July/early August, much later than the empirically observed average swarm date (May) in the dataset the model parameters were based on (Fig 1). The model also predicts that all drones should emerge in May and June, rather than continue to emerge into August as they do in empirically observed data (Fig 2).

Scenario 2

When we allow drone mating success to depend on the swarm date of the focal colony, we find that drone production and swarm timing in the focal colony overlap (Fig 3). Colonies are also predicted to swarm earlier, in May, closer to the time of swarming observed in empirical data (Fig 3). The model predicts two peaks in drone emergence, one before and one after swarming (Fig 2). Thus, we find that the constraint of requiring coordination between sexes changes the optimal timing of reproductive investment in temperate honey bees.

Discussion

Our results demonstrate that when the behavior of other colonies is assumed to follow current observed patterns for the timing of swarm and drone production, the optimal response for any individual colony is to segregate its investment in male and female reproductives in time, with males being produced first and queens second. The optimal colony-level solution would not be evolutionary stable at a population level because drones and queen would never co-occur and all colonies would have zero mating success if every colony adopted it. Selection to optimize the timing of reproduction in temperate honey bees must therefore be constrained by the need to coordinate the production of reproductives with other colonies in the population. In empirical observations, honey bee colonies do not segregate investments in drones and queens as the first model scenario suggests; instead drones are maintained throughout late spring and summer before and after swarming occurs (Lee and Winston 1987).

Under our second model scenario, we asked how the optimal reproductive timing changes when we assume the whole population is adopting the same strategy as the focal colony; under this assumption, the maximum queen availability occurs when the focal colony swarms. When all colonies mirror the focal colony, we find the optimal timing of swarming shifts earlier and drones persist over a longer period. In this scenario, the timing of drone and swarm production is a much closer match to empirically observed data. These results suggest that the selective pressures shaping the timing of reproductive investment may be best understood as a coordination game

similar to the Battle of the Sexes (Luce and Raiffa 1989). In this game, the action with the highest payoff for males differs from the action with the highest payoff for females. However, both sexes receive the lowest payoff if they fail to coordinate and take different actions. If drones and queens did not need to find each other, the external constraints of seasonal resource availability lead to different optimal timings for their production. However, the additional constraint of coordination leads to a compromise that would be suboptimal based on external resource constraints alone.

Because the present model focuses on optimizing the behavior of an individual colony, it does not capture the evolutionary dynamics that occur if every colony is allowed to adopt any strategy of reproductive timing. In reality, there may be other types of equilibria other than all colonies adopting the same strategy, which would require a game theoretic model to reveal. By assuming all colonies in the population have the same set of constraints, the present model also ignores inter-colony variability, which, in reality, likely impacts reproductive decisions. For instance, larger colonies generally invest more in drones than small colonies because they can better afford to support them (Smith et al. 2014). It is possible that colonies adopt condition-dependent strategies of reproductive investment, which would affect the mating success of other colonies (Trivers and Willard 1973). This variability does not alleviate the need for coordination between colonies in the timing of male and female reproductives; rather colonies that overproduce drones relative to the population average will experience selection to coordinate the timing of their drone production to match the timing of queen production in colonies overproducing females. However, ignoring variability may

partly explain the slight mismatch between the empirically measured drone timing and that predicted by our model.

The current model allows us to consider ultimate selective pressures governing how a honey bee colony could globally optimize its resource allocation among drones and workers to maximize its annual reproductive success if workers have perfect knowledge of resource conditions and of current and future behavior of all members of the colony. In reality, workers operate on local and likely imperfect information about the colony's internal state and external conditions. Workers may therefore be under selection to locally maximize current reproductive success without regard to how current behavior affects future allocation decisions. To understand how locally triggered decisions would differ from globally optimal outcomes, however, would require consideration of the quality of potential proximate cues that might allow local information to provide insight into the same externalities that govern the global system. Regardless, there will still be an important coordination constraint acting on non-optimal, locally maximal outcomes.

We have employed a novel computational method for examining optimal reproductive timing in swarm-founding social insects. Our approach makes testable predictions about the optimal timing of resource allocation on colony growth and reproduction, given a limited season for resource gathering. Our results also reveal a fundamental, but previously overlooked, tradeoff between the energetically optimal time to invest in males in terms of maximizing overall colony growth and the need to

invest in males in ways that shape colony growth such that that drone and swarm (hence queen) production will co-occur.

Though not the focus of the present work, this method also provides a novel methodology for testing predictions about investment sex ratio in swarm founding social insects. This approach has the advantage of not needing to account for what fraction of workers should be considered indirect investments in males versus investments in the swarm. Instead, worker number is optimized directly, considering its effect on both male and female elements of reproductive success. A similar computational approach could also be applied more broadly to organisms that reproduce both sexually and by fission at the organismal level. Future work will hopefully apply this linear programming approach to understanding the timing of life history events in a variety of taxa, and a diversity of forms of reproductive investment.

Methods

We develop a linear programming model where the objective function to be maximized is colony fitness. We model a single colony for one season, where the colony is the decision-making agent and the variables to be manipulated include the number of worker eggs and drone eggs to rear each day, and the number of adult drones to eject from the colony each day. All other variables are deterministic, linear functions of those three variables (Table 1). Because colony activity occurs on a daily cycle, we use a series of discrete time difference equations to model colony growth. We assume that:

- The colony's ability to rear new bees is limited by the amount of food available to feed them and by the queen's egg laying capacity.
- Brood rearing is not limited by nurse bees because the colony appropriately allocates adult workers between nursing and foraging (Beshers et al. 2001).
- Adult workers have a net positive energy contribution and adult drones a net negative contribution, since they take energy to feed.
- Any energy not used each day is stored as honey. The colony can use more energy than it produces each day as long as there is enough honey to make up the deficit.
- Colonies produce one prime swarm each year and one afterswarm g days later.
- Colonies start with an average spring size B_1 and workers live an average number of days l_w.
- Drones live until the workers eject them from the colony but experience reproductive senescence with age.
- Drone mating success depends on the number of available queens and all drones have an equal chance of mating.

Although multiple theories exist about what proximal cues cause colonies to swarm (Winston et al. 1991; Grozinger et al. 2014), we assume the ultimate cause of swarming is the colony reaching reproductive stability, i.e. where the amount of food available to rear new workers exceeds the queen's capacity to lay them (Fefferman and

Starks 2006). To determine when it is optimal for the colony to swarm, we define the swarm date as t^* and solve the model to find the optimal value of the objective function. We then run the model for all possible values of t^* within 1..T. We define the optimal swarm date as the value of t^* which maximizes the optimal value of the objective function.

Model constraints

If we define $D_{t,s}$ as the number of s day old drones in the colony on day t and E_t as the number of drone eggs laid on day t , then $D_{t,1} = E_{t-e_d}$ where e_d is the number of days for a drone to develop to adulthood. We define $D_{t,s} = D_{t-1,s-1} - K_{t-1,s-1}$ where $K_{t,s}$ is the number of s day old drones ejected from the colony after day t. For each day t, $K_{t,s} \leq D_{t,s}$, i.e. the colony cannot kick out more drones than it has. If B_t is the number of adult workers in the colony on day t, H_t is the number of worker eggs laid on day t, e_w is the number of days for a worker to develop to adulthood, and l_w is the average lifespan of a worker (from egg to death), then $B_t = B_{t-1} + H_{t-e_w} - H_{t-l_w}$. If we define t^* as the day on which the first swarm issues from the parent colony, $(t^* + g)$ as the day the second swarm issues from the colony, and sf_d as the fraction of workers that go with swarm d, $B_{t^*} = (1 - sf_1)B_{t-1} + H_{t-e_w} - H_{t-l_w}$ and $B_{t^*+g} = (1 - sf_2)B_{t-1} + H_{t-e_w} - H_{t-l_w}$.

We assume for all days t, $B_t \geq B_{\min}$ where B_{min} is the critical size for the colony to stay alive. The colony energy budget is a function of the number of workers in the colony and the amount of energy used to rear workers, rear drones, and feed drones.

For each day t, $F_t = F_{t-1} + n_t B_t - m\sum_{\forall_s} D_{t,s} - pH_t - oE_t$ where F_t is the amount of honey stored in the colony, n_t is the net daily energy contribution of a worker, m is the daily energy consumed by a drone, p is the energy needed to rear a worker until eclosion, and o is the energy needed to rear a drone to eclosion. The net daily energy contribution per worker is an inverse parabolic function of day t such that: $n_t = \frac{n_{int} - n_y}{n_x^2}(t - n_x)^2 + n_y$ (see Supplementary Information Table S1 and Fig S9). In addition, the honey stored in the colony cannot exceed a maximum storage capacity v, i.e. $F_t \leq v$. The total number of worker and drone eggs laid each day cannot exceed the maximum laying rate of the queen, r_{max}, i.e. $H_t + E_t \leq r_{\max}$. For q days after swarming, H_t and E_t = 0(Winston 1987). On the day of swarming, the colony must meet reproductive stability, i.e. $\frac{n_{t^*}B_{t^*} - mD_{t^*} - oE_{t^*}}{p} \geq r_{\max}$.

At the end of the active season, the parent colony must be of sufficient size, B_{minT}, and have sufficient honey stores, F_{min}, to survive winter, i.e. $B_T \geq B_{\min\mathrm{T}}$ and $F_T \geq F_{\min}$. Swarms grow at a deterministic rate; their final size depends on their initial size and date of issue. If $S_{d,t}$ is the number of workers in swarm d on day t and $G_{d,t}$ is the number of worker eggs in swarm d on day t, $S_{d,t} = S_{d,t-1} + G_{d,t-e_w} - G_{d,t-l_w}$ and $G_{d,t} = \min\left(r_{max}, \frac{n_t S_{d,t}}{p}\right)$.

We define $O_{t,s}$, the expected number of offspring produced by an s day old drone on day t, as the product of $c_{t,s}$, the probability of an s day old drone mating on day t, and w_t, the final size of an average swarm issued on day t, i.e. $O_{t,s} = c_{t,s}w_t$. The value w_t is calculated deterministically for each day t using the same growth parameters as swarm

growth and $c_{t,s}$ is taken from the empirical literature (Lee and Winston 1987). Drones are assumed to reach maturity at age 12 days and experience reproductive senescence after maturity, where $c_{t,s}$ is a linearly decreasing function of s (Winston 1987).

Comparison with/without coordination between sexes

We compared two alternative scenarios in the model. In Scenario 1, the expected drone mating probability on day t, $c_{t,s}$, is proportional to the empirically observed number of queens available on day t, as reported by Lee & Winston (Lee and Winston 1987); in this data set, available queens were present from May to late July with the peak occurring in late May (see Fig S1). In this scenario, $c_{t,s}$ is independent of the day the focal colony swarms, i.e. other colonies in the population produce swarms independent of the choices of the focal colony (Fig S1).

Without any other constraints, we would expect all colonies in the population to evolve toward the optimal swarm date. If the focal colony behaves optimally by swarming on a different day than everyone else, there will be no stable evolutionary equilibrium because other colonies could do better by swarming later or earlier. In Scenario 2, we asked how the optimal times for a colony to swarm, and to produce drones, changes if all colonies in the population swarm on the same day as the focal colony, i.e. if colonies coordinate the production of reproductives. To model this scenario, we assumed the peak value of $c_{t,s}$ always occurs on day t_d, the day the focal colony swarms, while keeping the shape of $c_{t,s}$ the same as in Lee & Winston (Lee and Winston 1987).

Model objective function

The objective to be maximized is the sum of all offspring colonies plus the original parent colony times the size of each colony, where size is a proxy for colony survival probability (Lee and Winston 1987). The value of each offspring colony in the objective function is discounted by its relatedness to the workers of the parent colony. We assume the workers control the level of investment in drone and worker brood since workers can control which larvae are reared through selective feeding and brood cannibalism (Crailsheim 1990). Our objective is therefore defined as: maximize: $k_1\left(\sum_{\forall s,t} O_{t,s}D_{t,s}\right) + k_3(B_T) + k_2\left(S_{1,T}\right) + k_3\left(S_{2,T}\right)$, where k_1 is relatedness to workers in drone-fathered colonies, k_2 is relatedness among workers in the mother queen-led colony, and k_3 is the relatedness to workers in daughter queen-led colonies. For each day t, we define $t^* = t$ and find the optimal solution. Then for all possible values of t^*, we define the optimal swarm date to be the value t^* which produces the maximum value of the objective function.

Tables

Table 1. Full list of model variables.

Variable name	Variable meaning
E(t)	Number of drone eggs laid in colony on day *t*
D(t,s)	Number of *s* day old drones in colony on day *t*
K(t,s)	Number of *s* day old drones ejected from colony after day *t*
H(t)	Number of worker eggs laid in colony on day *t*
B(t)	Number of adult workers in colony on day *t*
S(1,t)	Number of workers in prime swarm on day *t*
S(2,t)	Number of workers in afterswarm on day *t*
G(d,t)	Number of worker eggs laid in swarm *d* on day *t*
F(t)	Amount of food (in g honey) stored in parent colony on day *t*

Figure Legends

Figure 1. Queen and drone production do not co-occur in optimal solution. The dashed line shows the optimal number of drones and the solid line shows the optimal number of workers present in the colony on each day under model scenario 1. Under the assumption that other colonies in the population produce queens at an empirically estimated time (Lee and Winston 1987) regardless of whether it is optimal, we find the optimal swarm date for the focal colony to be the end of July. In this model scenario, the optimal behavior is for the colony to produce all drones between April and July and evict all drones before the colony swarms at the end of July. The dotted line shows the number of workers in the prime swarm on each day; the dash-dot line shows the number of workers in the afterswarm on each day in the optimal solution. The letters P and A indicate when the prime swarm and afterswarm, respectively, are produced in the model. The letter E indicates the empirically measured swarm date in mid-May (Lee and Winston 1987), much earlier than predicted by the model.

Figure 2. The timing of drone emergence as predicted by the model vs. empirically observed. (A) Under model scenario 1, we assume that all other colonies in the population produce queens at an empirically estimated time (Lee and Winston 1987). We find the optimal solution is for the parent colony to produce all drones early in the spring between March and May, earlier than empirically observed. (B) Under model scenario 2, we assume that all colonies in the population produce queens and swarm on

the same days as the focal colony; in other words, all colonies follow the same optimal timing. Under this assumption, the optimal solution is to produce a first pulse of drones from late April to late May and produce a second pulse of drones in June. (C) The bottom panel shows the empirically observed number of drones emerging each day (Lee and Winston 1987). Model scenario 2 predicts that colonies should start producing drones later and stop producing drones earlier than empirically observed but captures the empirically observed pattern of two distinct peaks in drone emergence.

Figure 3. Swarming earlier is optimal if drone mating success is tied to swarm date. In model scenario 2, we assume that all colonies in the population adopt the same optimal timing of drone and swarm production, i.e. the focal colony optimizes the timing of drone and swarm production given that all colonies in the population produce queens and swarm at the same time as the focal colony. Under this set of assumptions, we find the optimal swarm date is in late May/early June and the optimal behavior is to maintain drones from April to late May, evict all drones just before swarming, and maintain a second wave of drones from June to August. The solid line shows the optimal number of workers in the parent colony on each day, the dashed line shows the optimal number of drones on each day, the dotted line shows the number of workers in the prime swarm and the dash-dot line shows the number of workers in the afterswarm. The letters P and A indicate when the prime swarm and afterswarm, respectively, are produced in the model. The letter E indicates the empirically measured swarm date (Lee

and Winston 1987). The optimal swarm date predicted by model scenario 2 is much closer to the empirically observed date than that predicted by model scenario 1.

Figures

Figure 1

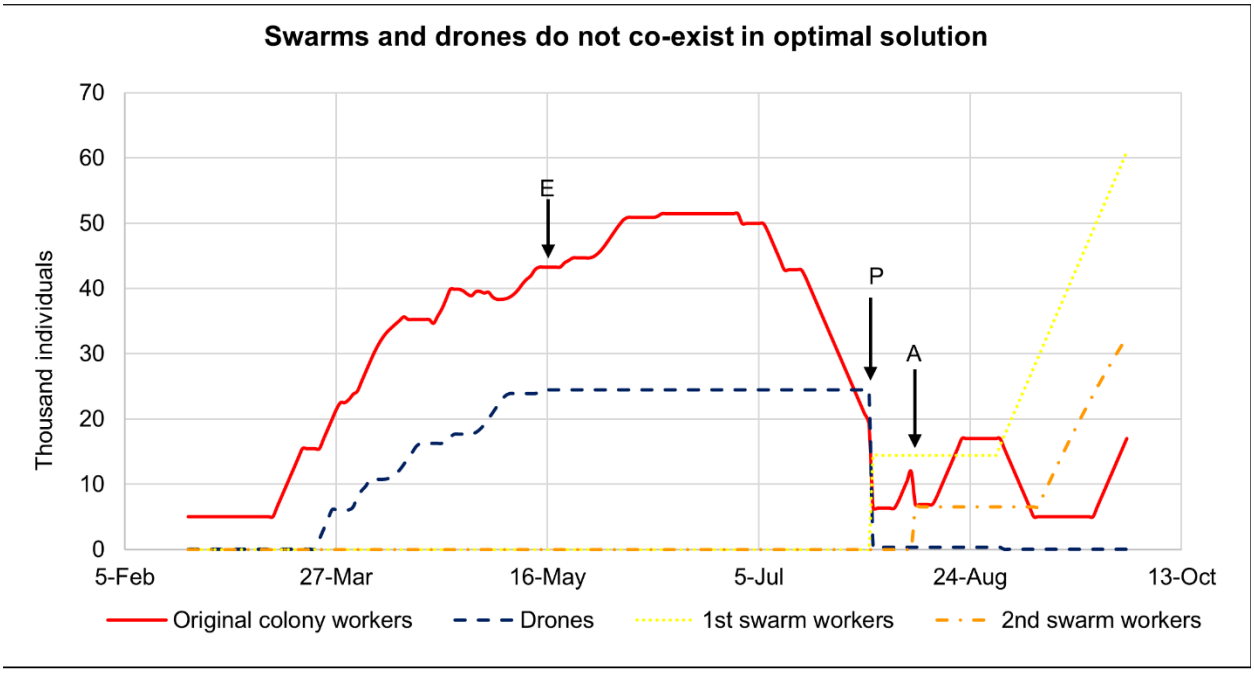


Figure 2

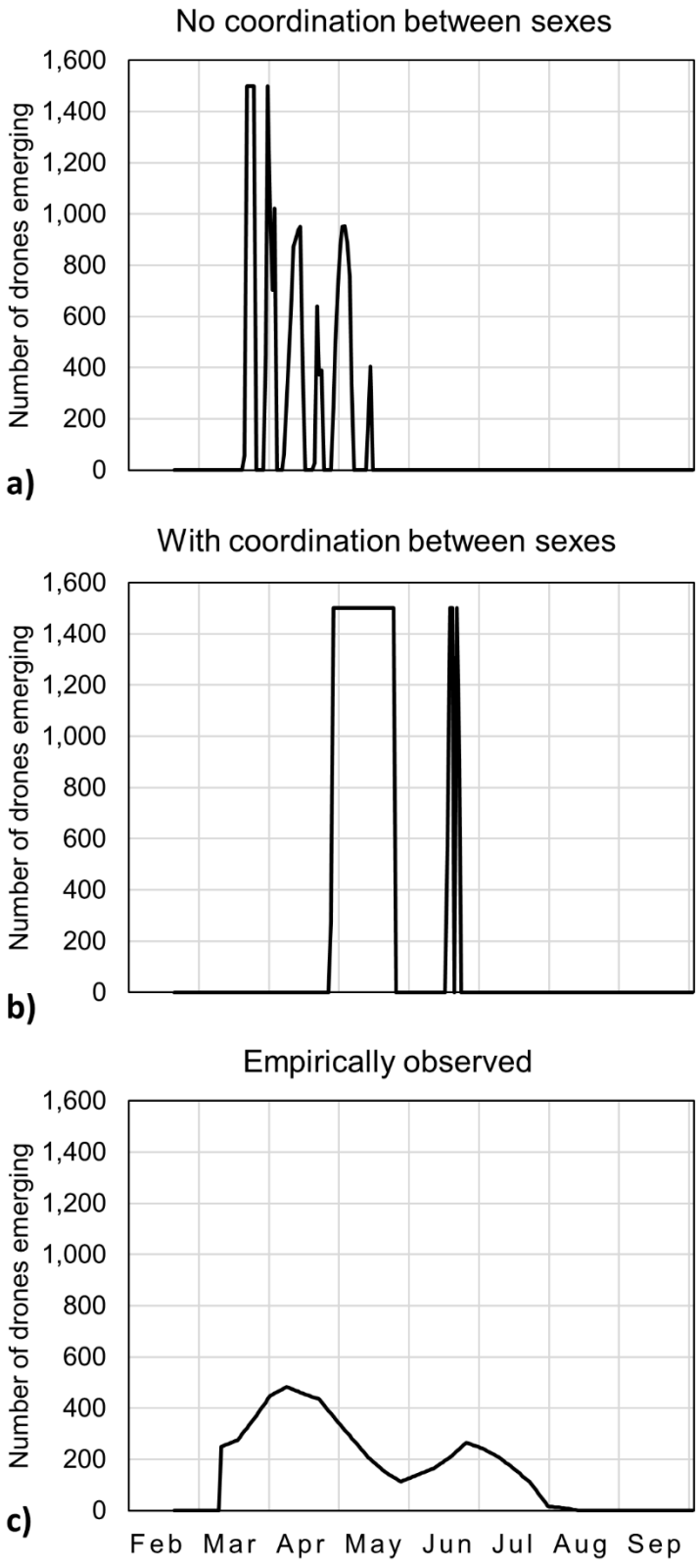
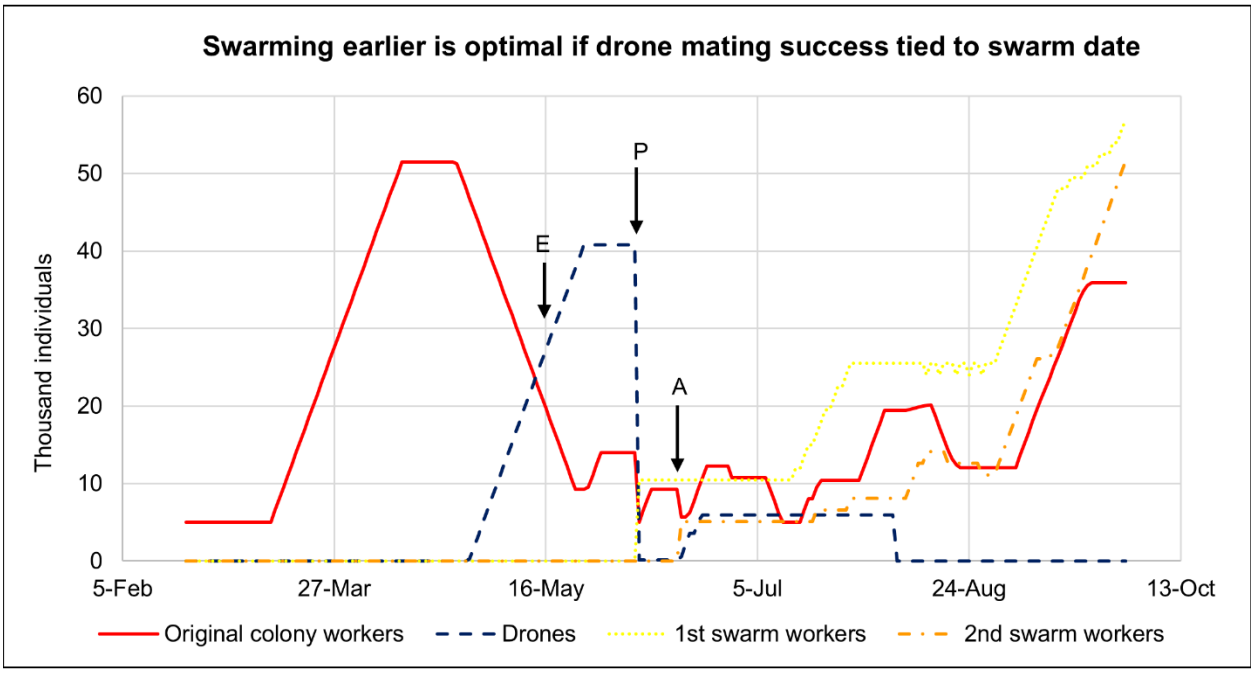

No coordination between sexes
Number of drones emerging
1,600
1,400
1,200
1,000
800
600
400
200
0
a)
With coordination between sexes
Number of drones emerging
1,600
1,400
1,200
1,000
800
600
400
200
0
b)
Empirically observed
Number of drones emerging
1,600
1,400
1,200
1,000
800
600
400
200
0
c)
Feb Mar Apr May Jun Jul Aug Sep

Figure 3


Swarming earlier is optimal if drone mating success tied to swarm date
Thousand individuals
60
50
40
30
20
10
0
E
P
A
5-Feb
27-Mar
16-May
5-Jul
24-Aug
13-Oct
Original colony workers
Drones
1st swarm workers
2nd swarm workers

Supplementary Information for Chapter 3

Figure S1

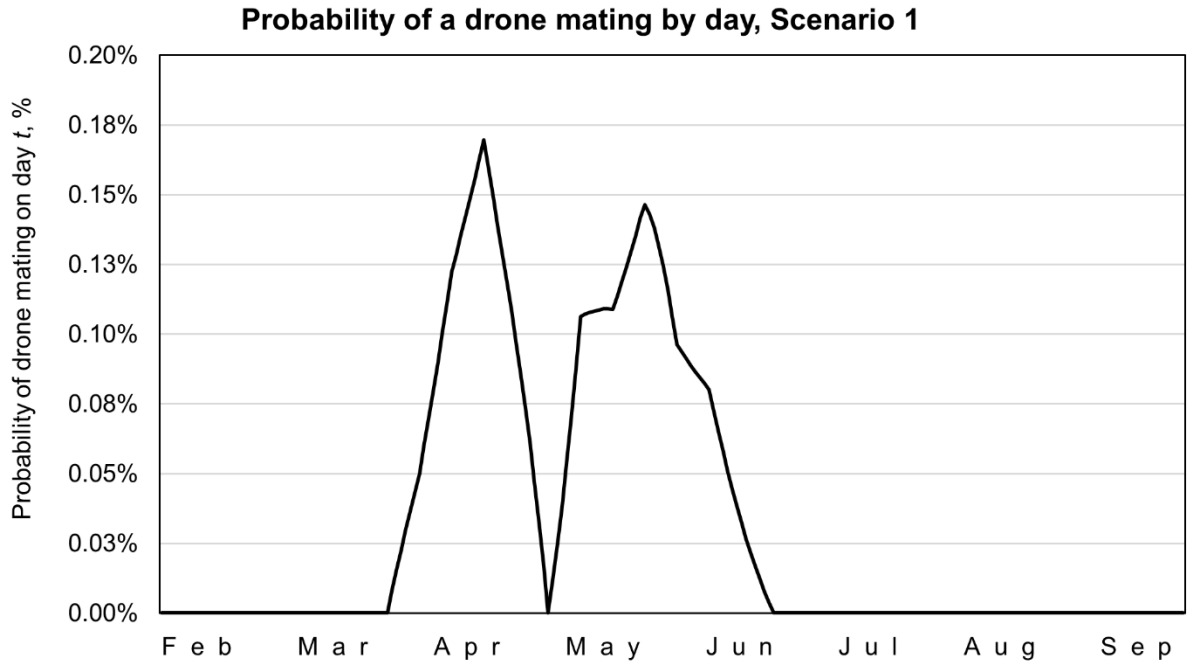


Figure S1. Probability of a drone mating each day based on empirically estimated queen numbers. Under model scenario 1, we assumed that the probability of a drone mating on each day is a function of the number of queens produced by other colonies in the population on that day, as estimated empirically (Lee and Winston 1987). The number of available queens has two peaks, one in April and one in late May. We assumed for simplicity that queens are available for mating on the day swarms are produced.

Figure S2

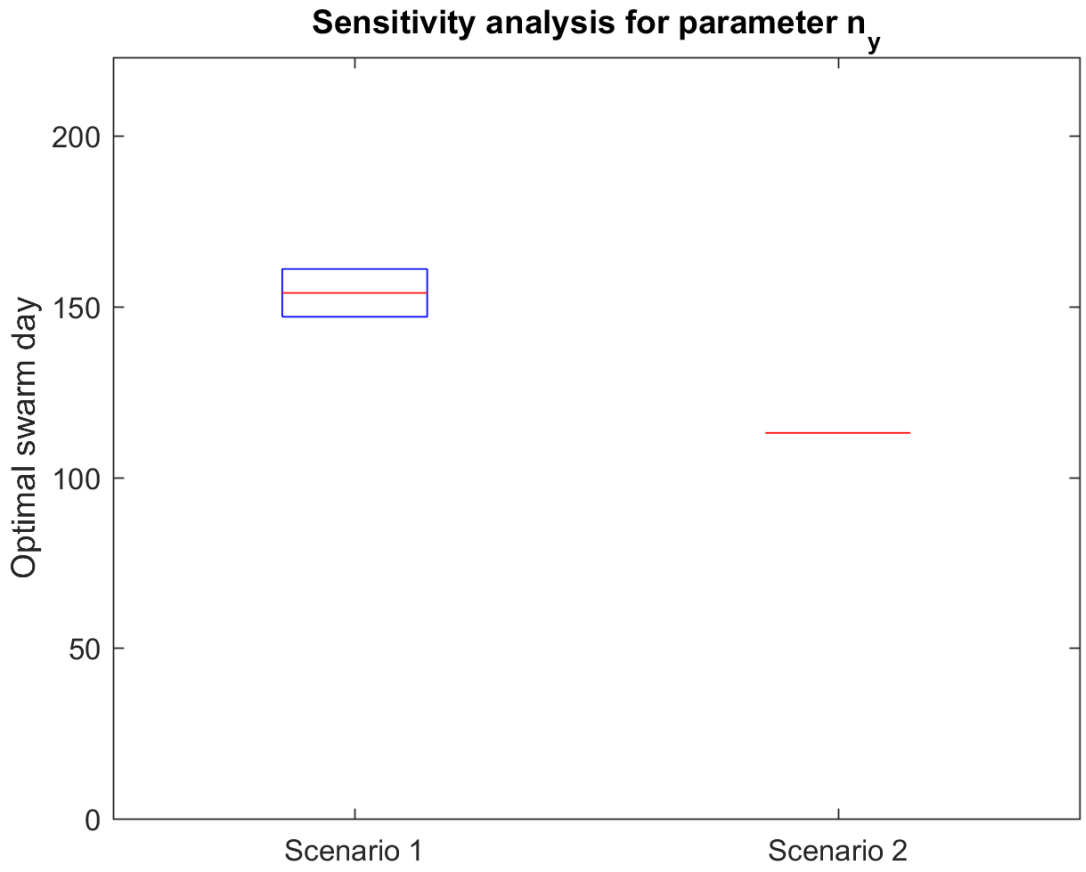


Figure S2. Sensitivity analysis for net worker daily energy contribution. We performed a sensitivity analysis to examine how our estimate of parameter n_y, the net daily energy contribution of a worker, influences the model outcome. We examined the original value and a range of +/- 10%. The low-end value results in no feasible solution to the optimization problem because worker productivity is too low for the colony to sustain itself.

Figure S3

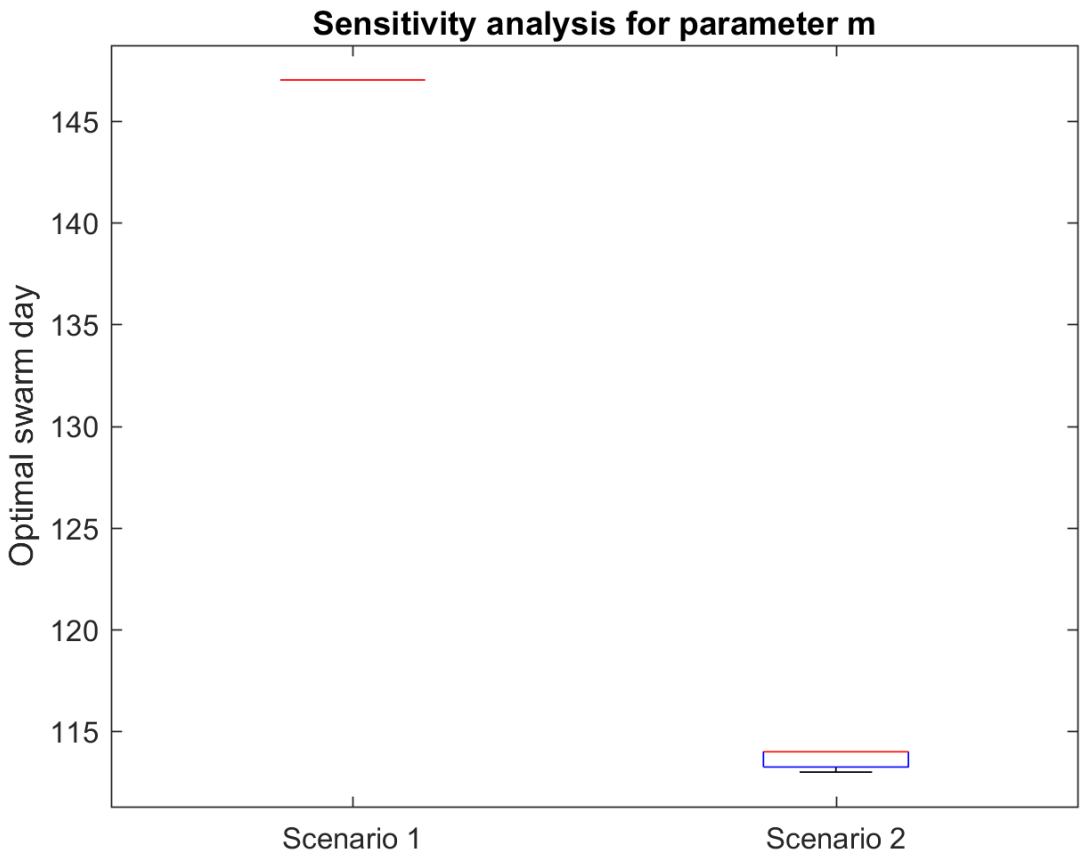


Figure S3. Sensitivity analysis for daily drone energy consumption. We performed a sensitivity analysis to examine how our estimate of parameter *m*, the daily energy consumption of a drone, influences the optimal timing of swarming under our two model scenarios. We examined the original value and a range of +/- 10%. Within this range, the exact value of *m* has little effect on the optimal swarm time.

Figure S4

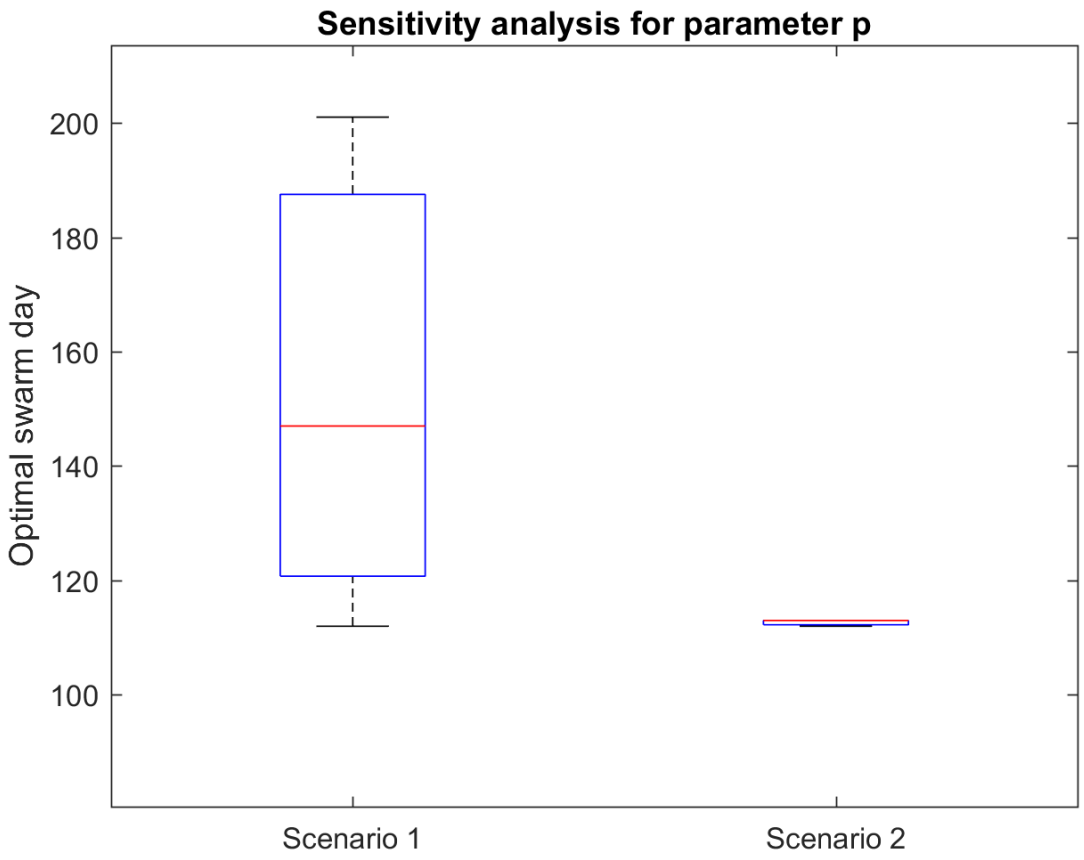


Figure S4. Sensitivity analysis for energy needed to rear a worker. We performed a sensitivity analysis to examine how our estimate of parameter *p*, the energy needed to rear a worker to adulthood, affects the optimal timing of swarming. We examined the original value and a range of +/- 10%. While, we still find a different optimal behavior between our two model scenarios, the magnitude of the difference does depend on the energetic cost of producing workers.

Figure S5

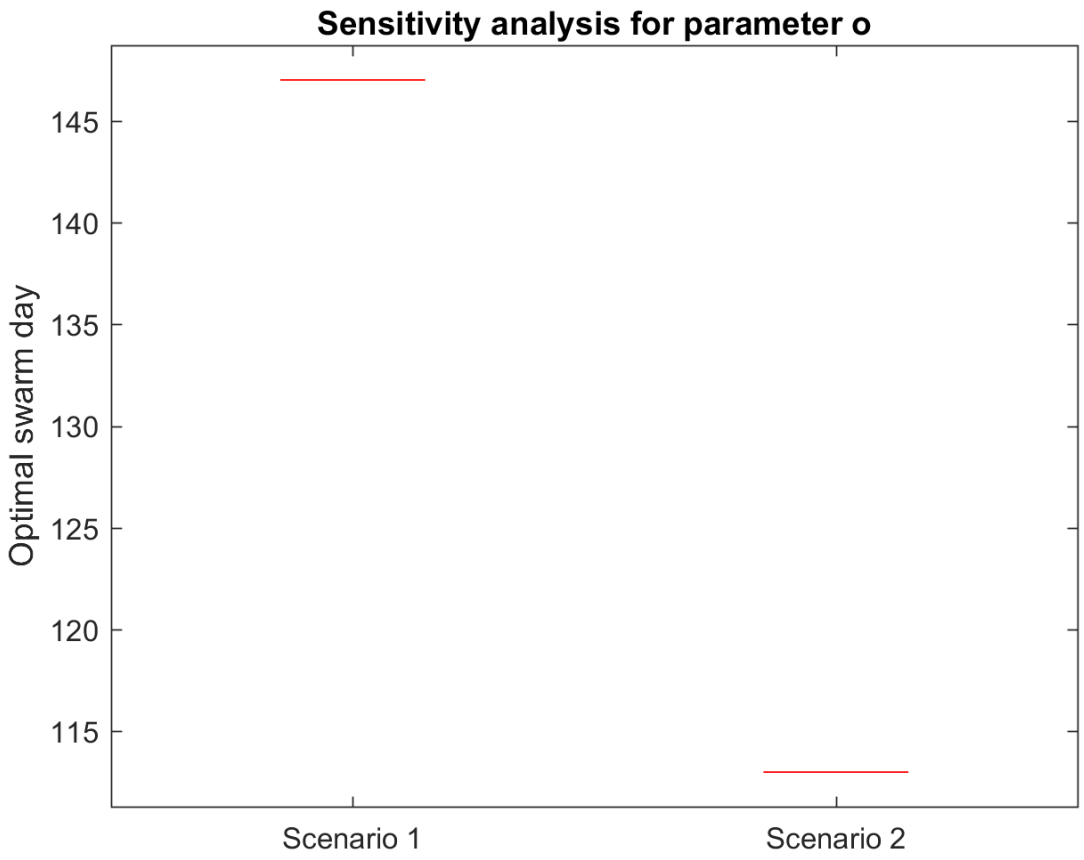


Figure S5. Sensitivity analysis for energy needed to rear a drone. We performed a sensitivity analysis to examine how our estimate of parameter *o*, the energy needed to rear a drone to adulthood, affects the optimal timing of swarming. We examined the original value and a range of +/- 10%. Within this range, we find little effect of the energetic cost of producing drones on the optimal solution. This makes sense given that the main cost of producing a drone is the need to support it for as long as it remains in the colony; the initial cost to rear a drone to adulthood is relatively small relative to maintenance costs.

Figure S6

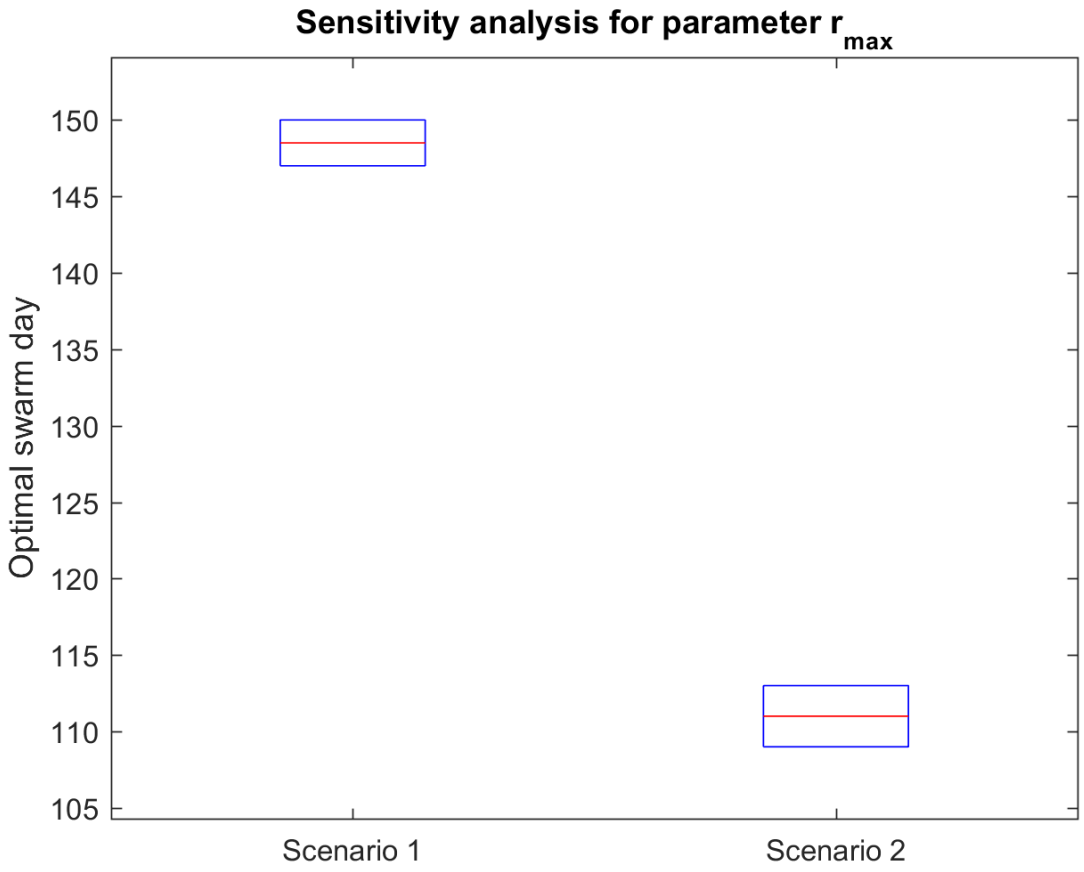


Figure S6. Sensitivity analysis for queen's maximum laying capacity. We performed a sensitivity analysis to examine how our estimate of parameter r_{max}, the maximum number of eggs the queen can lay per day, affects the optimal swarm date. We examined the original value and a range of +/- 10%. On the low end of this range, the model has no feasible solution. In the parameter range where there is a feasible solution, the exact value of r_{max} has little effect on the optimal swarm date.

Figure S7

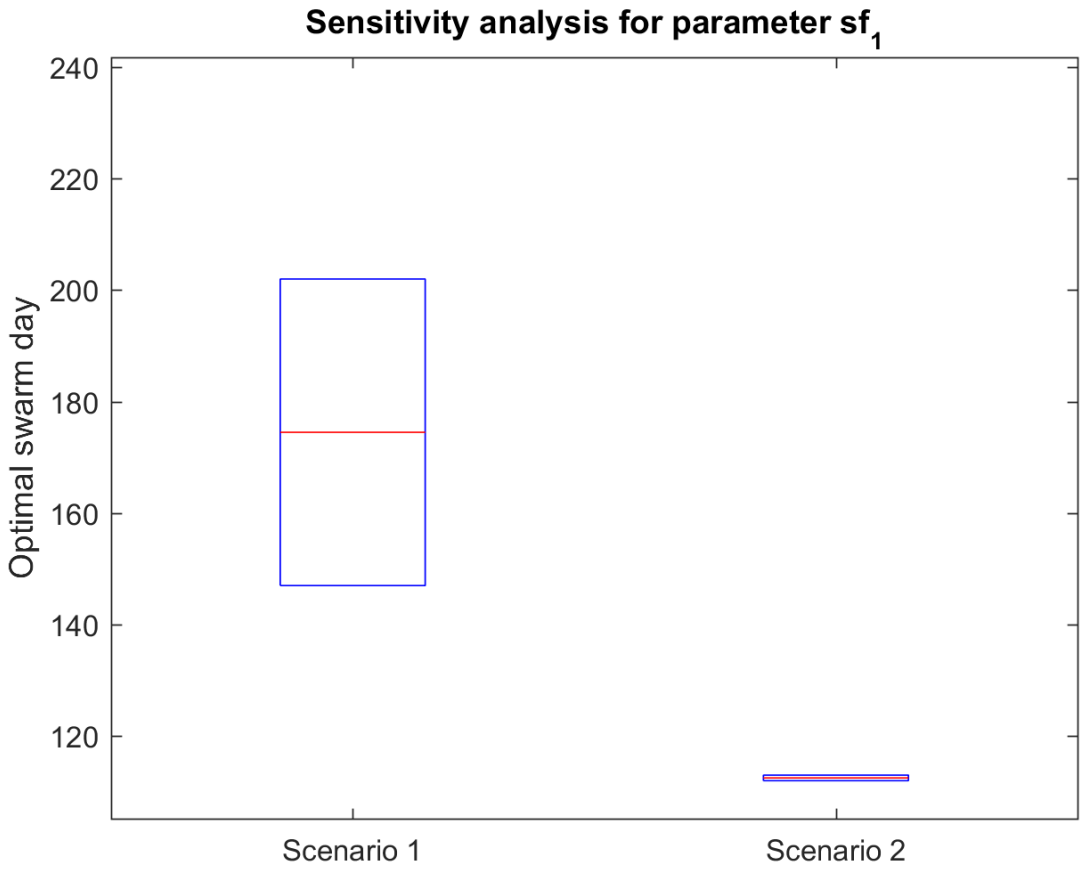


Figure S7. Sensitivity analysis for fraction of colony leaving with swarm. We performed a sensitivity analysis to examine how our estimate of parameter sf_1, the fraction of the worker population leaving with the prime swarm, affects the model outcome. We examined the original value and a range of +/- 10%. On the high end of this range, the model has no feasible solution because there are not enough workers remaining in the parent colony to sustain it. Within the range where there is a feasible solution, there remains a difference in optimal swarm time between model scenarios 1 and 2, although the magnitude of the difference depends on the size of the swarm fraction.

Figure S8

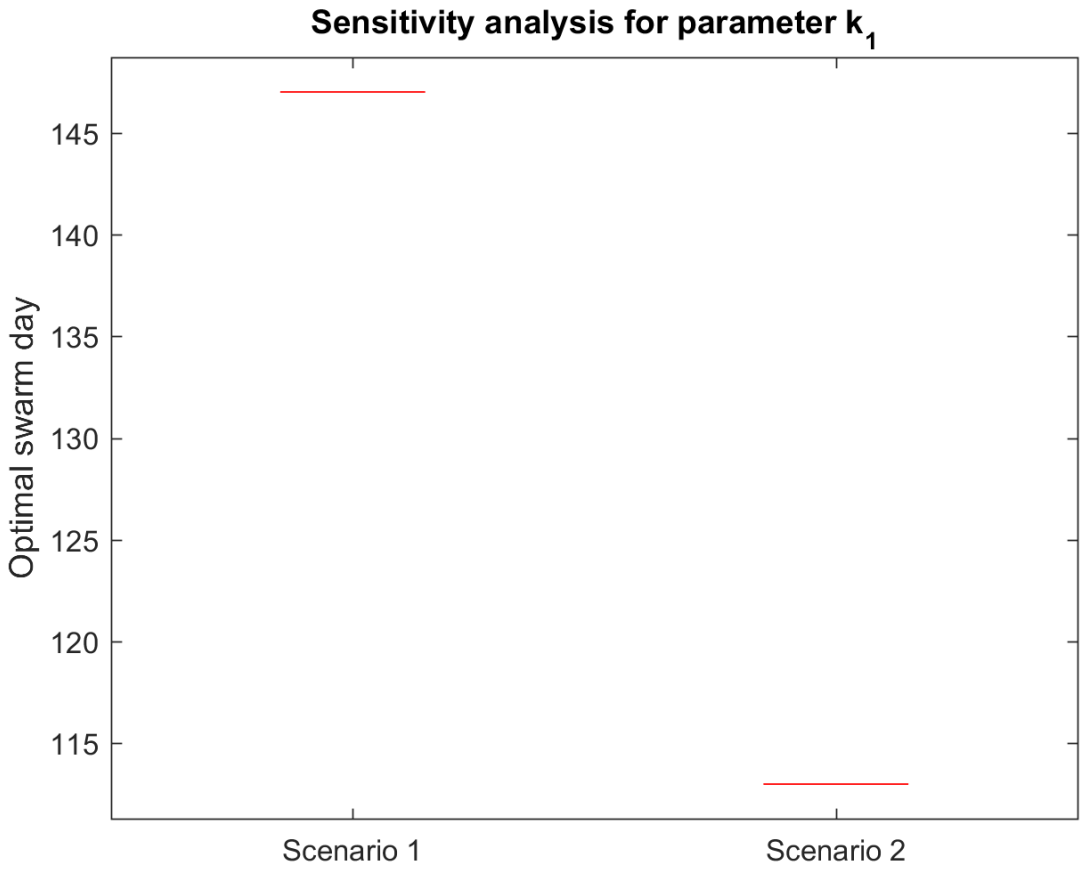


Figure S8. Sensitivity analysis for relatedness of workers to drone-fathered colonies. We performed a sensitivity analysis to examine how parameter k_1, the relatedness of workers in the original colony to the colonies fathered by drones, affects the model results. We examined a parameter range of +/- 10%; within this range, relatedness had no impact on the optimal time of swarming for either scenario 1 or 2.

Figure S9

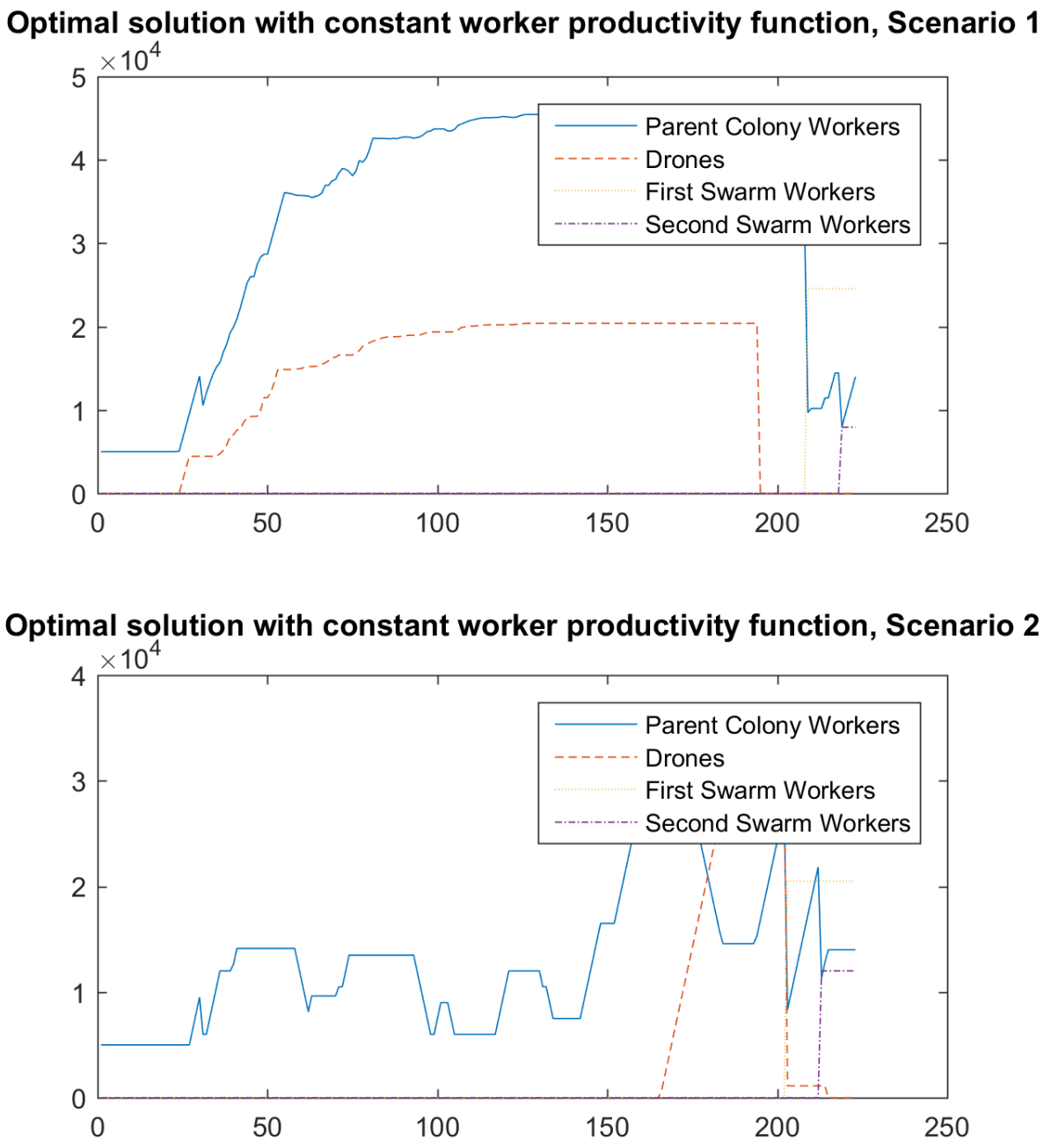


Figure S9. Effect of worker productivity function on optimal swarm time. We examined the effect of the functional response chosen for n_t, the net daily energy contribution of a worker on day t. In the main paper, we used an inverse quadratic function, assuming

available resources increase throughout the spring and then decrease again in fall. We also examined the effect of constant resource availability (panels A and B). Panel A shows the optimal solution under model Scenario 1, in which we assumed only the focal colony swarms on the optimal day. Panel B shows the optimal solution under model Scenario 2, in which all colonies in the population swarm on the optimal day.

Table S1. Full list of model parameters and their values.

Parameters		Value	Source
T	Number of days considered in model	223	Estimated
n_x	Worker daily energy contribution x maximum	112	(Winston 1987; USEPA 2012)
n_y	Worker daily energy contribution y maximum	0.0444	(Winston 1987; USEPA 2012)
n_{int}	Worker daily energy contribution y intercept	0.0111	(Winston 1987; USEPA 2012)
m	Daily energy consumption per drone	.0665	(Winston 1987; USEPA 2012)
p	Energy needed to rear a worker to adulthood	.351	(Winston 1987)
o	Energy needed to rear a drone to adulthood	.142	(Winston 1987)
e_d	Days needed for drone to develop from egg to adult	24	(Winston 1987)
e_w	Days needed for worker to develop from egg to adult	21	(Winston 1987)
l_w	Worker life span (from egg to death)	52	(Winston 1987)
r_{max}	Max number of eggs queen can lay per day	1,500	(Winston 1987)
v	Max amount of honey that can be stored in colony at a time	50,000	(Seeley 1977)
$B_{min\ T}$	Min workers parent colony must have at end of active season	14,000	(Winston 1987)
F_{min}	Min honey parent colony must have at end of active season	20,000	(Winston 1987)
B_{min}	Min workers parent colony must have for all days to remain viable	5,000	(Winston 1987)
k_1	Average relatedness of workers in parent colony to drone-fathered colonies	0.02083	(Winston 1987)

k_2	Average relatedness of workers in parent colony to each other	0.29167	(Winston 1987)
k_3	Average relatedness of workers in parent colony to daughter queen-founded colonies	0.14583	(Winston 1987)
w_t	Final size of an average swarm produced on day t		Calculated
$c_{t,s}$	Probability of an s day old drone mating on day t		(Lee and Winston 1987)
B_1	Initial number of workers in parent colony on day 1	5,000	(Winston 1987)
F_1	Initial honey stored in parent colony on day 1	5,000	Estimated
sf_1	Fraction of workers leaving with prime swarm	.75	(Rangel and Seeley 2012)
sf_2	Fraction of workers leaving with afterswarm	.55	(Rangel and Seeley 2012)
g	Number of days between issue of prime swarm and afterswarm	10	(Winston 1987)
q	Days needed for a new queen to start producing eggs	8	(Winston 1987)

CONCLUDING REMARKS

Social organisms are under different constraints and face different selective pressures than solitary organisms. For advanced eusocial organisms like the honeybee, the colony is more like a functionally integrated superorganism, with shared resources and a shared fitness, than a collection of individuals (Seeley 1989; Wilson and Holldobler 2005). Trade-offs over resource allocation occur at the level of the colony and life history traits evolve in response to selection on the colony phenotype, an emergent property of the interacting phenotypes of its members, rather than on individual traits directly.

My dissertation research provides a quantitative framework for examining how selection on colony-level resource allocation shapes life history traits in eusocial insects. I have used this framework to gain insight into ecological factors driving the evolution of aging and reproduction in honeybees. Like many organisms, honeybees depend on resources whose availability vary in space and time (Seeley and Visscher 1985). A colony experiences predictable seasonal changes in the ability to acquire food resources (Seeley and Visscher 1985) as well as in the risk of accidental mortality to workers in the process of resource acquisition (Dukas 2008). These environmental fluctuations result in seasonal changes in the selection on worker senescence.

Since colonies have limited energetic resources, seasonal changes in the force of selection on worker senescence result in changes in the colony's optimal level of investment in worker maintenance. We would expect colonies to invest more in workers

during times of lower extrinsic mortality or lower resource availability, when their durability has the greatest impact on colony function. Optimal allocation to worker maintenance is also influenced by how efficient maintenance is (i.e. what gains in worker longevity can be achieved for a given cost) and the relationship between investment and lifespan. For instance, diminishing returns on investment (in terms of increased worker durability) should select for lower investment in worker maintenance. Worker physiological constraints thus interact with environmental constraints to shape a colony's optimal resource allocation.

Seasonal environmental constraints are also important in shaping a honeybee colony's reproductive investment. The costs and benefits of allocation to growth and reproduction change over the course of the limited season during which resource acquisition and reproduction can occur. Since only female offspring inherit a fraction of the worker population, the trade-off over allocation between somatic growth and reproduction also influences the way reproductive investments are allocated between the sexes. The optimal timing of reproductive investment in honeybees is influenced by a combination of inter-colony sexual conflict and energetic trade-offs between growth and reproduction.

While in the studied system, reproduction by swarming produces the observed cost asymmetry between the sexes, it is nonetheless the case that coordination of the timing of fertility and constraints from seasonal fluctuation in the viability of offspring may affect reproductive investment decisions in many social systems. These results

therefore contribute to a broader understanding of how conflicting selective pressures can interact to shape reproductive allocation in social organisms.

Although taking a colony-level view of life history evolution provides valuable insights, it is important to note that even in advanced eusocial societies, there is the potential for conflict among group members over resource allocation. Honeybee workers are not truly sterile and under some circumstances can lay male eggs; the resolution of intra-group conflict over male production has been discussed extensively by others (Francis L. W. Ratnieks 1988; Visscher 1996; Pirk et al. 2004; Wenseleers et al. 2004). However, since worker reproduction is rare in insects with advanced eusociality, it is reasonable to assume worker contribution to colony success is more important in driving life history evolution.

Eusocial insects include many species of great ecological and economic importance, including pollinators (Southwick and Southwick 1992; Calderone 2012), predators (Ronauer 2009), and ecosystem engineers (Folgarait 1998; Jouquet et al. 2011). Better understanding the selective forces driving life history evolution in eusocial organisms is thus a matter of practical concern as well as an important conceptual advance to life history theory. My dissertation research has focused on life history evolution in honeybees, a model system that has received much empirical study due to their importance to humans and the ease of keeping captive colonies. However, many of these results could easily be applied to other eusocial insects and have practical consequences for either managing species of conservation concern (e.g Colla and Packer

2008) or combating species with detrimental effects on humans and natural ecosystems (e.g. Tsutsui and Suarez 2003).

It would be an interesting direction for future empirical study to examine the predictions of these models in other species with similar social organization as honeybees that are under different ecological constraints to further validate conclusions about how ecological constraints shape life history in social systems. Furthermore, it is my hope that this theoretical framework can be applied more broadly to extend our understanding of life history theory evolution beyond the level of the individual.

REFERENCES

Abrams, P. A. 1993. Does increased mortality favor the evolution of more rapid senescence? Evolution 47:877–887.

Abrams, P. A., O. Leimar, S. Nylin, and C. Wiklund. 1996. The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. The American Naturalist 147:381–395.

Abrams, P. A., and D. Ludwig. 1995. Optimality theory, Gomperts' law and the disposable soma theory of senescence. Evolution 49:1055–1066.

Alaux, C., F. Ducloz, D. Crauser, and Y. Le Conte. 2010. Diet effects on honeybee immunocompetence. Biology Letters 6:562–565.

Amdam, G. V., A. L. T. O. Aase, S.-C. Seehuus, M. K. Fondrk, K. Norberg, and K. Hartfelder. 2005. Social reversal of immunosenescence in honey bee workers. Experimental Gerontology 40:939–947.

Amdam, G. V., and S. W. Omholt. 2002. The regulatory anatomy of honeybee lifespan. Journal of Theoretical Biology 216:209–28.

Amdam, G. V., O. Rueppell, M. K. Fondrk, R. E. Page, and C. M. Nelson. 2009. The nurse's load : Early-life exposure to brood-rearing affects behavior and lifespan in honey bees (*Apis mellifera*). Experimental Gerontology 44:467–471.

Amdam, G. V., Z. L. P. Simões, A. Hagen, K. Norberg, K. Schrøder, Ø. Mikkelsen, T. B. L. Kirkwood, et al. 2004. Hormonal control of the yolk precursor vitellogenin regulates immune function and longevity in honeybees. Experimental Gerontology 39:767–73.

Austad, S. N. 1993. Retarded senescence in an insular population of Virginia opossums (*Didelphis virginiana*). Journal of Zoology 229:695–708.

Austad, S. N., and K. E. Fischer. 1991. Mammalian aging, metabolism, and ecology: Evidence from the bats and marsupials. Journal of Gerontology 46:B47–B53.

Baer, B. 2005. Sexual selection in *Apis* bees. Apidologie 36:187–200.

Bateson, M. 2007. Recent advances in our understanding of risk-sensitive foraging preferences. Proceedings of the Nutrition Society 61:509–516.

Baudisch, A. 2005. Hamilton's indicators of the force of selection. Proceedings of the National Academy of Sciences 102:8263–8268.

———. 2011. The pace and shape of ageing. Methods in Ecology and Evolution 2:375–382.

Bérubé, C. H., M. Festa-Bianchet, and J. T. Jorgenson. 1999. Individual differences, longevity, and reproductive senescence in bighorn ewes. Ecology 80:2555–2565.

Beshers, S. N., Z. Y. Huang, Y. Oono, and G. E. Robinson. 2001. Social inhibition and the regulation of temporal polyethism in honey bees. Journal of Theoretical Biology 213:461–79.

Bulmer, M. G. 1983. Sex ratio theory in social insects with swarming. Journal of Theoretical Biology 100:329–339.

Calderone, N. W. 2012. Insect pollinated crops, insect pollinators and US agriculture: Trend analysis of aggregate data for the period 1992-2009. PLoS ONE 7:24–28.

Caswell, H. 1989. Matrix Population Models: Construction, Analysis, and Interpretation. Sinauer Associates (1st ed.). Sinauer Associates, Sunderland, MA.

Caswell, H. 2000. Prospective and retrospective perturbation analyses: their roles in conservation biology. Ecology 81:619–627.

Caswell, H. 2007. Extrinsic mortality and the evolution of senescence. Trends in Ecology & Evolution 22:173–174.

Chen, H. Y., and A. A. Maklakov. 2012. Longer life span evolves under high rates of condition-dependent mortality. Current Biology 22:2140–2143.

Chippindale, A. K., A. M. Leroi, S. B. Kim, and M. R. Rose. 1993. Phenotypic plasticity and selection in *Drosophila* life-history evolution. I. Nutrition and the cost of reproduction. Journal of Evolutionary Biology 6:171–193.

Cichoń, M. 1997. Evolution of longevity through optimal resource allocation. Proceedings of the Royal Society of London B: Biological Sciences 264:1383–1388.

Cichoń, M., and J. Kozłowski. 2000. Ageing and typical survivorship curves result from optimal resource allocation. Evolutionary Ecology Research 2:857–870.

Colla, S. R., and L. Packer. 2008. Evidence for decline in eastern North American bumblebees (Hymenoptera: Apidae), with special focus on *Bombus affinis Cresson*. Biodiversity and Conservation 17:1379–1391.

Crailsheim, K. 1990. The protein balance of the honey bee worker. Apidologie 21:417–429.

Crailsheim, K., L. H. W. Schneider, N. Hrassnigg, G. Bühlmann, U. Brosch, R. Gmeinbauer, and B. Schöffmann. 1992. Pollen consumption and utilization in worker honeybees (*Apis mellifera carnica*): Dependence on individual age and function. Journal of Insect Physiology 38:409–419.

Demetrius, L. 1978. Adaptive value, entropy and survivorship curves. Nature 275:213–214.

Dowling, D. K. 2012. Aging: Evolution of life span revisited. Current Biology 22:947–949.

Dukas, R. 2008. Mortality rates of honey bees in the wild. Insectes Sociaux 55:252–255.

Fefferman, N. H., and P. T. Starks. 2006. A modeling approach to swarming in honey bees (*Apis mellifera*). Insectes Sociaux 53:37–45.

Fluri, P., H. Wille, L. Gerig, and M. Luscher. 1977. Juvenile hormone, vitellogenin and haemocyte composition in winter worker honeybees (*Apis mellifera*). Experientia 33:1240–1241.

Folgarait, P. 1998. Ant biodiversity to ecosystem functioning: A review. Biodiversity and Conservation 7:1121–1244.

Foster, D. J., and R. V Cartar. 2011. What causes wing wear in foraging bumble bees? The Journal of Experimental Biology 214:1896–1901.

Fourer, R., D. M. Gay, and B. W. Kernighan. 2003. AMPL: a modeling language for mathematical programming. Thomson/Brooks/Cole.

Francis L. W. Ratnieks. 1988. Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. The American Naturalist 132:217–236.

Franks, N. R., and B. Hölldobler. 1987. Sexual competition during colony reproduction in army ants. Biological Journal of the Linnean Society 30:229–243.

Gaillard, J.-M., D. Allaine, D. Pontier, N. G. Yoccoz, and D. E. L. Promislow. 1994. Senescence in natural populations of mammals: A reanalysis. Evolution 48:509–516.

Gardner, S. N., and M. Mangel. 1997. When can a clonal organism escape senescence? The American Naturalist 150:462–490.

Gasser, M., M. Kaiser, D. Berrigan, and S. C. Stearns. 2000. Life-history correlates of evolution under high and low adult mortality. Evolution 54:1260–1272.

Gilley, D. C. 1998. The identity of nest-site scouts in honey bee swarms. Apidologie 29:229–240.

Grozinger, C. M., J. Richards, and H. R. Mattila. 2014. From molecules to societies: Mechanisms regulating swarming behavior in honey bees (*Apis spp.*). Apidologie 45:327–346.

Hamilton, W. D. 1966. The moulding of senescence by natural selection. Journal of Theoretical Biology 12:12–45.

Hamilton, W. D. 1967. Extraordinary sex ratios. Science 156:477–488.

Healy, K., T. Guillerme, S. Finlay, A. Kane, S. B. A. Kelly, D. Mcclean, D. J. Kelly, et al. 2014. Ecology and mode-of-life explain lifespan variation in birds and mammals. Proceedings of the Royal Society B 281.

Hölldobler, B., M. Lindauer, and N. R. Franks. 1985. Reproduction, foraging efficiency and worker polymorphism in army ants. Experimental Behavioral Ecology and Sociobiology: In Memoriam Karl von Frisch,1886-1982 91–107.

Holmes, D. J., and S. N. Austad. 1994. Fly now, die later: Life-history correlates of gliding and flying in mammals. Journal of Mammalogy 75:224–226.

Jouquet, P., S. Traoré, C. Choosai, C. Hartmann, and D. Bignell. 2011. Influence of termites on ecosystem functioning. Ecosystem services provided by termites. European Journal of Soil Biology 47:215–222.

Keller, L. 1998. Queen lifespan and colony characteristics in ants and termites. Insectes Sociaux 45:235–246.

Keller, L., and M. Genoud. 1997. Extraordinary lifespans in ants: a test of evolutionary theories of ageing. Nature 389:958–960.

Khoury, D. S., A. B. Barron, and M. R. Myerscough. 2013. Modelling food and population dynamics in honey bee colonies. PloS one 8:e59084.

Kirkwood, T. B. 1977. Evolution of aging. Nature 270:301–304.

———. 2010. Why can't we live forever. Scientific American 303:42–49.

Kirkwood, T. B., and S. N. Austad. 2000. Why do we age? Nature 408:233–238.

Kirkwood, T. B., and M. R. Rose. 1991. Evolution of senescence: late survival sacrificed for reproduction. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 332:15–24.

Kozłowski, J. 1993. Measuring fitness in life-history studies. Trends in Ecology & Evolution 8:84–85.

Kramer, B. H., and R. Schaible. 2013. Life span evolution in eusocial workers: A theoretical approach to understanding the effects of extrinsic mortality in a hierarchical system. PLoS ONE 8:e61813.

Kronenberg, F., and H. C. Heller. 1982. Colonial thermoregulation in honey bees (*Apis mellifera*). Journal of Comparative Physiology 148:65–76.

Lande, R. 1982. A quantitative genetic theory of life history evolution. Ecology 63:607–615.

Law, R. 1979. Optimal life histories under age-specific predation. The American Naturalist 114:399–417.

Lee, P. C., and M. L. Winston. 1987. Effects of reproductive timing and colony size on the survival, offspring colony size and drone production in the honey bee (*Apis mellifera*). Ecological Entomology 12:187–195.

Lee, R. D. 2003. Rethinking the evolutionary theory of aging: transfers, not births, shape senescence in social species. Proceedings of the National Academy of Sciences 100:9637–42.

Luce, R. D., and H. Raiffa. 1989. Games and decisions: Introduction and critical survey.

Dover Publications, New York, NY.

Macevicz, S. 1979. Some consequences of Fisher's sex ratio principle for social hymenoptera that reproduce by colony fission. The American Naturalist 113:363–371.

Mangel, M., and S. B. Munch. 2005. A life-history perspective on short- and long-term consequences of compensatory growth. The American Naturalist 166:E155–E176.

Mangel, M., J. A. Rosenheim, and F. R. Adler. 1994. Clutch size, offspring performance, and intergenerational fitness. Behavioral Ecology 5:412–417.

McNamara, J., and A. Houston. 1980. The application of statistical decision theory to animal behaviour. Journal of Theoretical Biology 85:673–690.

McNamara, J. M., and K. L. Buchanan. 2005. Stress, resource allocation, and mortality. Behavioral Ecology 16:1008–1017.

Medawar, P. B. 1952. An unsolved problem of biology. An Inaugural Lecture Delivered at University College London, Inaugural lecture. H.K. Lewis and Company.

Metz, J. A. J., S. D. Mylius, and O. Diekmann. 2008. When does evolution optimize? Evolutionary Ecology Research 10:629–654.

Miller, J. K. 2001. Escaping senescence: Demographic data from the three-toed box turtle (*Terrapene carolina triunguis*). Experimental Gerontology 36:829–832.

Mitesser, O., N. Weissel, E. Strohm, and H. J. Poethke. 2007. Optimal investment allocation in primitively eusocial bees: A balance model based on resource limitation of the queen. Insectes Sociaux 54:234–241.

Morbey, Y. E., C. E. Brassil, and A. P. Hendry. 2005. Rapid senescence in pacific salmon. The American Naturalist 166:556–568.

Münch, D., and G. V Amdam. 2010. The curious case of aging plasticity in honey bees. FEBS letters 584:2496–503.

Münch, D., C. D. Kreibich, and G. V Amdam. 2013. Aging and its modulation in a long-lived worker caste of the honey bee. The Journal of Experimental Biology 216:1638–49.

Munch, S. B., and M. Mangel. 2006. Evaluation of mortality trajectories in evolutionary biodemography. Proceedings of the National Academy of Sciences 103:16604–16607.

Nesse, R. M. 1988. Life table tests of evolutionary theories of senescence. Experimental Gerontology 23:445–53.

Nesse, R. M. 2005. Natural selection and the regulation of defenses: A signal detection analysis of the smoke detector principle. Evolution and Human Behavior 26:88–105.

Omholt, S. W., and G. V Amdam. 2004. Epigenetic regulation of aging in honeybee workers. Science of Aging Knowledge Environment 2004:pe28.

Oster, G. F., and E. O. Wilson. 1978. Caste and Ecology in the Social Insects. Monographs in Population Biology. Princeton University Press.

Page, R. E., and R. A. Metcalf. 1984. A population investment sex ratio for the honey bee (*Apis mellifera L.*). The American Naturalist 124:680–702.

Page, R. E., and C. Y. Peng. 2001. Aging and development in social insects with emphasis on the honey bee, *Apis mellifera L*. Experimental Gerontology 36:695–711.

Partridge, L., and K. Fowler. 1992. Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. Evolution 46:76–91.

Partridge, L., N. Prowse, and P. Pignatelli. 1999. Another set of responses and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. Proceedings of the Royal Society Biological Sciences 266:255–261.

Perry, C. J., E. Søvik, M. R. Myerscough, and A. B. Barron. 2015. Rapid behavioral maturation accelerates failure of stressed honey bee colonies. Proceedings of the National Academy of Sciences 112:3427–32.

Peterson, M. 2009. An Introduction to Decision Theory. Cambridge Introductions to Philosophy. Cambridge University Press.

Pianka, E. R. 1970. On r- and K-Selection. The American Naturalist 104:592–597.

Piraino, S., F. Boero, B. Aeschbach, and V. Schmid. 1996. Reversing the life cycle: Medusae transforming into polyps and cell transdifferentiation in *Turritopsis nutricula* (Cnidaria, Hydrozoa). The Biological Bulletin 190:302–312.

Pirk, C. W. W., P. Neumann, R. Hepburn, R. F. A. Moritz, and J. Tautz. 2004. Egg viability and worker policing in honey bees. Proceedings of the National Academy of Sciences 101:8649–8651.

Poitrineau, K., O. Mitesser, and H. J. Poethke. 2009. Workers, sexuals, or both? Optimal allocation of resources to reproduction and growth in annual insect colonies. Insectes Sociaux 56:119–129.

Promislow, D. E. L., and P. H. Harvey. 1990. Living fast and dying young: A comparative analysis of life-history variation among mammals. Journal of Zoology 220:417–437.

Rangel, J., and T. D. Seeley. 2012. Colony fissioning in honey bees: Size and significance of the swarm fraction. Insectes Sociaux 59:453–462.

Remolina, S. C., D. M. Hafez, G. E. Robinson, and K. A. Hughes. 2007. Senescence in the worker honey bee *Apis mellifera*. Journal of Insect Physiology 53:1027–33.

Reznick, D. 2002. r - and K - selection revisited: the role of population regulation in life-history evolution. Ecology 83:1509–1520.

Reznick, D. N., M. J. Bryant, D. Roff, C. K. Ghalambor, and D. E. Ghalambor. 2004. Effect of extrinsic mortality on the evolution of senescence in guppies. Nature 431:1095–1100.

Reznick, D., L. Nunney, and A. Tessier. 2000. Big houses, big cars, superfleas and the costs of reproduction. Trends in Ecology and Evolution 15:421–425.

Ricklefs, R. E. 2010. Life-history connections to rates of aging in terrestrial vertebrates. Proceedings of the National Academy of Sciences 107:10314–9.

Robinson, G. E., R. E. Page, C. Strambi, and A. Strambi. 1989. Hormonal and genetic control of behavioral integration in honey bee colonies. Science 246:109–112.

Ronauer, D. J. C. K. 2009. Recent advances in army ant biology (Hymenoptera: Formicidae). Myrmecological News 12:51–65.

Rose, M. R. 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. Evolution 38:1004–1010.

Rueppell, O., C. Bachelier, M. K. Fondrk, and R. E. Page. 2007. Regulation of life history determines lifespan of worker honey bees (*Apis mellifera L.*). Experimental Gerontology 42:1020–32.

Sakagami, S. F., and H. Fukuda. 1968. Life tables for worker honeybees. Researches in Population Ecology X:127–139.

Santos-Filho, P. de S., D. D. A. Alves, A. Eterovic, V. L. Imperatriz-Fonseca, and A. de M. P. Kleinert. 2006. Numerical investment in sex and caste by stingless bees (Apidae: Meliponini): A comparative analysis. Apidologie 37:207–221.

Seehuus, S.-C., K. Norberg, U. Gimsa, T. Krekling, and G. V Amdam. 2006. Reproductive protein protects functionally sterile honey bee workers from oxidative stress. Proceedings of the National Academy of Sciences 103:962–7.

Seeley, T. 1977. Measurement of nest cavity volume by the honey bee (*Apis mellifera*). Behavioral Ecology and Sociobiology 2:201–227.

Seeley, T. D. 1989. The honey bee colony as a superorganism. American Scientist 77:546–553.

———. 1995. The Wisdom Of The Hive. Harvard University Press.

———. 1997. Honey bee colonies are group-level adaptive units. The American Naturalist 150:S22–S41.

Seeley, T. D., and P. K. Visscher. 1985. Survival of honeybees in cold climates: the critical timing of colony growth and reproduction. Ecological Entomology 10:81–88.

Smedal, B., M. Brynem, C. D. Kreibich, and G. V Amdam. 2009. Brood pheromone suppresses physiology of extreme longevity in honeybees (*Apis mellifera*). The Journal of Experimental Biology 212:3795–801.

Smith, C. C., and S. D. Fretwell. 1974. The optimal balance between size and number of offspring. The American Naturalist 108:499–506.

Smith, M. L., M. M. Ostwald, J. C. Loftus, and T. D. Seeley. 2014. A critical number of workers in a honeybee colony triggers investment in reproduction. Naturwissenschaften 101:783–790.

Sohal, R. S., and R. Weindruch. 1996. Oxidative stress, caloric restriction, and aging. Science 273:59–63.

Solis, C. R., C. R. Hughes, C. J. Klingler, J. E. Strassmann, and D. C. Queller. 1998. Lack of kin discrimination during wasp colony fission. Behavioral Ecology 9:172–176.

Southwick, E. E., and L. Southwick. 1992. Estimating the economic value of honey bees (Hymenoptera: Apidae) as agricultural pollinators in the United States. Journal of Economic Entomology 85:621–633.

Stearns, S. C. 1976. Life-history tactics: a review of the ideas. The Quarterly Review of Biology 51:3–47.

Stearns, S. C., M. Ackermann, M. Doebeli, and M. Kaiser. 2000. Experimental evolution of aging, growth, and reproduction in fruitflies. Proceedings of the National Academy of Sciences 97:3309–3313.

Tarpy, D. R., D. C. Gilley, and T. D. Seeley. 2004. Levels of selection in a social insect: A review of conflict and cooperation during honey bee (*Apis mellifera*) queen replacement. Behavioral Ecology and Sociobiology 55:513–523.

Tarpy, D. R., M. Simone-Finstrom, and T. A. Linksvayer. 2016. Honey bee colonies regulate queen reproductive traits by controlling which queens survive to adulthood. Insectes Sociaux 63:169–174.

Trivers, R. L., and H. Hare. 1976. Haploidploidy and the evolution of the social insect. Science 191:249–263.

Trivers, R. L., and D. E. Willard. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90–92.

Tsutsui, N. D., and A. V Suarez. 2003. The colony structure and population biology of invasive ants. Conservation Biology 17:48–58.

USEPA. 2012. White Paper in Support of the Proposed Risk Assessment Process for Bees. Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, Environmental Fate and Effects Division, Washington, DC; Environmental Assessment Directorate, Pest Management Regulatory Agency, Health Canada, Ottawa, Canada; California D.

van Noordwijk, A. J., and G. de Jong. 1986. Acquisition and allocation of resources: Their influence on variation in life history tactics. The American Naturalist 128:137–142.

Vaupel, J. W., A. Baudisch, M. Dölling, D. A. Roach, and J. Gampe. 2004. The case for negative senescence. Theoretical Population Biology 65:339–351.

Visscher, P. K. 1996. Reproductive conflict in honey bees: a stalemate of worker egg-laying and policing. Behavioral Ecology and Sociobiology 39:237–244.

Wenseleers, T., H. Helanterä, A. Hart, and F. L. W. Ratnieks. 2004. Worker reproduction and policing in insect societies: an ESS analysis. Journal of evolutionary biology 17:1035–47.

Wilkinson, G. S., and J. M. South. 2002. Life history, ecology and longevity in bats. Aging Cell 1:124–31.

Williams, G. C. 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution 11:398–411.

———. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. American Naturalist 100:687–690.

Williams, P. D., and T. Day. 2003. Antagonistic pleiotropy, mortality source interactions, and the evolutionary theory of senescence. Evolution 57:1478–1488.

Williams, P. D., T. Day, Q. Fletcher, and L. Rowe. 2006. The shaping of senescence in the wild. Trends in Ecology & Evolution 21:458–463.

Wilson, E. O., and B. Holldobler. 2005. Eusociality: Origin and consequences. Proceedings of the National Academy of Sciences 102:13367–13371.

Winston, M. L. 1987. The Biology of the Honey Bee. Harvard University Press.

Winston, M. L., H. A. Higo, S. J. Colley, T. Pankiw, and K. N. Slessor. 1991. The role of queen mandibular pheromone and colony congestion in honey bee (*Apis mellifera L.*) reproductive swarming (Hymenoptera: Apidae). Journal of Insect Behavior 4:649–660.

Winston, M. L., and C. D. Michener. 1977. Dual origin of highly social behavior among bees. Proceedings of the National Academy of Sciences 74:1135–7.