Demography in eusocial Hymenoptera

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"If you want to be incrementally better, be competitive.

If you want to be exponentially better, be cooperative."

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This thesis is based on the following four research articles, which are presented as chapters I to IV and are referred to in the introduction, the summary and the general discussion by their respective numbers:

Chapter I

Kramer, B.H., Schaible, R. (2013): Life Span Evolution in Eusocial Workers—A Theoretical Approach to Understanding the Effects of Extrinsic Mortality in a Hierarchical System. **PLoS ONE** 8(4).

Chapter II

Kramer, B.H., Scheuerlein, A.: Worker quality is determined by colony stage and not social environment in *Lasius niger*. Under review at **Myrmecological News.**

Chapter III

Kramer, B. H., Schaible, R. (2013): Colony size explains the life span differences between queens and workers in eusocial Hymenoptera. **Biological Journal of the Linnean Society,** 109 (3): 710-724.

Chapter IV

Kramer, B.H., Scharf, I., Foitzik, S.: Colony size and per-capita productivity in ants with single foragers. Under review at **Behavioral Ecology and Sociobiology.**

Summary

In my doctoral thesis I investigated the evolution of demographic traits within eusocial Hymenoptera. In the social bees, wasps and ants, eusociality has a unique effect on life span evolution as female larvae with the same genetic background can develop through phenotypic plasticity to a queen or a worker with vastly diverging life-history traits. Ant queens belong to the longest-lived insect species, while workers in most species live only a fraction of the queen's life span. The average colony size of a species is positively correlated with social complexity, division of labor and diverging morphological female phenotypes all of which also affect life span. Therefore the demographic traits of interest in this thesis were life span and colony size. To understand the evolution of worker life span I applied a trade-off model that includes both hierarchical levels important in eusocial systems, namely the colony- and the individual-level. I showed that the evolution of worker life span may be an adaptive trait on the colony level to optimize resource allocation and therefore fitness in response to different levels of extrinsic mortality. A shorter worker life span as a result of reduced resource investments under high levels of extrinsic mortality increases colony fitness. In a further study I showed that *Lasius niger* colonies produce different aging phenotypes throughout colony development. Smaller colonies which apply a different foraging strategy than larger colonies produced smaller workers, which in turn have a longer life span as compared to larger workers produced in larger colonies. With the switch to cooperative foraging in growing colonies individual workers become less important for the colony caused by their increasing redundancy. Alternatively a trade of between growth and life span may lead to the results found in this study. A further comparative analysis to study the effect of colony size on life span showed a correlation between queen and worker life span when colony size is taken into account. While neither worker nor queen life span was associated with colony size, the differences between queen and worker life span increase with larger average colony sizes across all eusocial Hymenoptera. As colony size affects both queen and worker life span, I aimed to understand which factors lead to the small colony sizes displayed by some ant species. I therefore analyzed per-capita productivity at different colony sizes of eight cavity dwelling ant species. Most colonies of the study species grew larger than optimal productivity predicted. Larger colony size was shown to increase colony homeostasis, the predictability of future productivity and in turn the survival probability of the colony. I also showed that species that deploy an individual foraging mode may circumvent the density dependent decline in foraging success by splitting the colony to several nest sites.

Zusammenfassung

In dieser Doktorarbeit habe ich die Evolution von demographischen Eigenschaften bei eusozialen Hautflüglern (Ameisen, Bienen und Wespen) untersucht. Eusozialität hat einen starken Einfluss auf die Evolution der Lebenspanne, aus einem weiblichen Ei können sich bei den sozialen Hautflüglern sowohl Königinnen als auch Arbeiterinnen Phänotypen entwickeln, die sich jedoch stark in ihrer Life-history unterscheiden. Ameisenköniginnen gehören zu den langlebigsten Insekten, aber leben Arbeiterinnen nur einen Bruchteil dieser Lebenspanne. Die durchschnittliche Koloniegröße ist hierfür ein bedeutender Faktor, denn sie steht in einem positiven Zusammenhang mit der Arbeitsteilung im Insektenstaat und den daraus resultierenden morphologischen Unterschieden zwischen den unterschiedlichen Kasten, die wiederum die Lebenspanne beeinflussen. Dementsprechend waren die Lebensspanne und die Koloniegröße die demographischen Eigenschaften auf die ich mich in dieser Doktorarbeit fokussiert habe. Um die Evolution der Lebenspanne der Arbeiterinnen zu untersuchen habe ich ein Trade-off Modell entwickelt, welches die hierarchische Struktur, die man bei sozialen Insekten findet, berücksichtigt. Ich konnte dadurch zeigen, dass die Investitionen in Arbeiterinnen bei hohem extrinsischem Sterberisiko reduziert werden sollten, um die Fitness der Kolonie zu optimieren. Interessanterweise ist in diesem Falle eine kürzere Arbeiterinnenlebenspanne mit einer höheren Fitness verbunden. In einer weiteren Studie habe ich gezeigt, dass während des Wachstums einer *Lasius niger* Kolonie die Investitionen in einzelne Arbeiterinnen variieren. Kleine Kolonien produzieren kleinere Arbeiterinnen mit einer längeren Lebensspanne als größere Kolonien. Die Unterschiede in der Lebensspanne können entweder durch den Größenunterschied oder aus den veränderten Anforderungen an die Arbeiterinnen resultieren, die mit der Änderung der furagier Strategie bei größeren Kolonien zusammenhängen. In einer weiteren, vergleichenden Studie habe ich dann den Effekt der Koloniegröße unterschiedlicher eusozialer Hautflügler auf die Lebensspanne untersucht. Während weder die Lebensspanne der Königinnen noch die der Arbeiterinnen mit der Koloniegröße im Bezug stehen, zeigt der Unterschied in der Lebenspanne zwischen diesen beiden Phänotypen einer Art eine positive Beziehung zur Koloniegröße. Um zu erklären, warum verschiedene Ameisenarten unterschiedlich große Kolonien bilden, habe ich bei acht Ameisenarten, die kleine Kolonien bilden, die Produktivität pro Arbeiterin untersucht. Es zeigte sich, dass diese pro-Kopf Produktivität, welche direkt die Fitness der Kolonie bestimmt, bei keiner der untersuchten Arten mit der Koloniegröße ansteigt. Eine Möglichkeit, die Reduktion der pro-Kopf Produktivität zu umgehen, ist das Aufteilen der Kolonie auf mehrere Nester, damit die gleiche Anzahl Arbeiterinnen auf eine größere Fläche verteilt ist.

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General Introduction

Demography

Demography is the statistical study of populations. It encompasses the size, structure and distribution of populations and its change due to birth, migration, aging and death. Thus, demography is a function of the individual parameters of growth, reproduction and death. In this thesis I exclusively focus on the measures mortality and colony size and their evolution in social insects.

Biodemography emerged as a discipline at the interface of biology and demography (Carey 2008) and is concerned with the demographic changes that occur in organisms over time and how these evolved forms are better adapted for coping with the demands of their environment (Carey, 2008). Most important for this work is that biodemography tries to combine the hierarchical organization inherent to biology and demography. Both biology and demography share an organization of different hierarchical levels (Carey 2008), and life itself exists as hierarchically nested levels of organization, were lower level units compose higher level units (Michod 2000). The different levels of importance in biology are the gene, genome, cell, tissue, multicellular organism and society or eusocial organisms (Heylighen 2000; Michod 2007). In demography the different levels are represented by individuals, cohorts and the population (Carey 2008). For the work on eusocial organisms this is of importance because the evolution of eusociality marks a major evolutionary transition where individuals join to form a larger entity, namely the colony (Smith and Szathmary 1997). This is why eusocial colonies have also been named superorganisms (Wheeler 1911; Hölldobler and Wilson 2009).

Eusociality

Eusociality represents the highest form of social organization and is characterized by overlapping adult generations, cooperative brood care and reproductive division of labor (Wilson 1971; Michener 1969). This definition was coined to cover social insects because initially eusociality was thought to occur only in the class Insecta and moreover, only in the orders Isoptera (including the termites) and the Hymenoptera (including ant, bee and wasp species). However, over the last twenty years the same organizational structures that define eusociality where found in other species such as aphids (Aoki 1987), ambrosia beetles (Kent and Simpson 1992), thrips (Crespi 1992), snapping shrimp (Duffy 1996) and mole-rats (Burda et al. 2000; Alexander et al. 1991).

In eusocial Hymenoptera, colonies are composed by one (monogyne) or several queens (polygynous) and a few to several million (Beckers et al. 1989) more or less sterile, female workers. Among the eusocial Hymenoptera, sex is determined by the haplodiploid sex determination system (Cook 1993). Males develop from unfertilized haploid eggs and females from fertilized diploid eggs. This system provides higher levels of genetic relatedness among workers (sisters) of a colony than between mother and daughter. This potential of high relatedness among members of a social insect colony and the occurrence of behaviorally or physiologically sterile workers has greatly influenced the theories, which explain the evolution of eusociality, namely the kin selection theory (also known as inclusive fitness), which states that individual can increase their fitness by helping related relatives to reproduce, (Hamilton 1964) and group selection (Allee 1951), which implies that eusociality evolved from cooperation between individuals and that selection is raised to the level of the group and acts between and within groups. Even Darwin stated that the occurrence of social insects could "actually be fatal to my whole theory" (Darwin 1860) and proposed that workers evolved through selection at the colony level. Up to today, the levels of selection in eusocial societies and during the evolution of eusociality are strongly debated (Nowak et al. 2011; Abbot et al. 2011). In this thesis I focus on evolutionary processes and their consequences that take place once eusociality was established and societies are bound by altruism and division of labor to form a higher entity, namely the colony (Gadau et al. 2009). The colony can thus be likened to the body of an individual organism. As a result of division of labor, the colony is composed of reproductive individuals (queens) that represent the germ line and workers that represent the soma, but as compared to a multicellular organism with lower genetic relatedness between its members which gives raise to conflict and competing levels of selection (individual- and colony-level) (Boomsma 2009; Ross and Matthews 1991; Wilson and Sober 1989; Wheeler 1911). Multilevel selection (Wilson 1997b, 1997a) (the modern version of group selection) does in fact account for the this different level of biological organization and thus the process of selection can be studied at those different levels (Damuth and Heisler 1988). Multilevel selection is often seen as the opposite of kin selection but in fact, both theories have been termed as two ways conceptualizing the same process of natural selection (Gardner and Foster 2008), since group selection as compared to kin selection also involves both direct and indirect benefits (Michod and Herron 2006). Ultimately the fitness of an individual within a colony depends on the reproductive success of the whole colony (Hamilton 1964; Woyciechowski and Kozlowski 1998).

The evolution of life span within social insects

Social insects are of particular interest for research on aging and on factors promoting the evolution of life span. Perennial social insect have evolved extraordinary life spans. Reproductive individuals (queens) may reach life span of more than 28 years (Keller and Genoud 1997) and within one species the difference in life span between reproductive queens and non –reproductive worker may be 100-fold (Page Jr and Peng 2001; Winston 1991;

Remolina and Hughes 2008). Most intriguing is the fact that both queen and worker could have been reared from the same female larvae thus they represent two different phenotypes of one genotype with distinct life-histories affecting life span and reproduction (Munch et al. 2008). The Phenotypes are triggered by different gene expression patterns activated by different environmental condition throughout larval development (Evans and Wheeler 1999; Hoffman and Goodisman 2007; Winston 1991) and differing developmental times (Remolina and Hughes 2008). Social insect queens are long lived in comparison to solitary insects and the combination of advanced sociality, high fecundity and long life span has evolved multiple times independently (Remolina and Hughes 2008). The same effects of eusociality have been found in eusocial mole-rats (Dammann and Burda 2006; Dammann et al. 2011), and the trend of diverging life span patterns is already evident throughout social evolution as shown in chapter III. Several studies focus on the evolution of queen life span in social insects (Heinze and Schrempf 2008; Keller and Genoud 1997) or on factors promoting long life span such as reproduction (Hartmann and Heinze 2003; Schrempf et al. 2005; Tsuji 1996; Tsuji et al. 1996) or the effects of the social environment (Munch et al. 2008). Life span of workers is of particular interest not only in comparison to the queen. Several factors are known to affect worker life span: seasonality (Fukuda and Sekiguchi 1966), social and demographic composition of the hive (Robinson 1992; Rueppell et al. 2009), task and activity (Robinson 1992; Rueppell et al. 2007a; Rueppell et al. 2007b) and caste and the associated levels of extrinsic mortality (Chapuisat and Keller 2002). Several studies suggest that senescence, an increase in the rate of mortality with age, is apparent in workers (Remolina et al. 2007; Rueppell et al. 2007a; Rueppell et al. 2007b). But explanations for the life span differences between queen and workers received little attention (Remolina and Hughes 2008) and are often explained solely by the differences in extrinsic mortality (Heinze and Schrempf 2008; Parker 2011; Schmid-Hempel and Wolf 1988).

Evolutionary (ultimate) and mechanistic (proximate) theories of aging are important to understand the evolution of life span in the social insects (Hughes and Reynolds 2005). Three evolutionary theories (mutation accumulation theory (Medawar 1952), agonistic pleiotropy (Williams 1957) and the disposable soma theory (Kirkwood 1977)) attempt to explain why aging patterns emerge and why aging has been stable in the course of evolution. In general, the evolutionary theories of aging explain that a reduction of the force of selection with increasing age leads to the accumulation of late acting deleterious mutations (Medawar 1952), or that early life benefits are traded off with late life disadvantages (Williams 1957; Kirkwood 1977).

More precisely, the mutation accumulation theory points out that most individuals die from parasites or predators before they reach old ages. Therefore selection pressure to maintain a functional organism is reduced at the ages where most individuals died from extrinsic sources of mortality. Thus, mutations which have negative effects only on survival late in life (when most individual of the population died from extrinsic sources) are likely to accumulate because selection pressure is low to remove those mutations from the genome. The agonistic pleiotropy theory is comparable to the mutation accumulation theory but implicates that mutations have pleiotrophic effects and that early life benefits of those mutations are traded off against their deleterious late life effects. The disposable soma theory argues that an organism must trade off limited resources for reproduction and physiological maintenance. If the investment in maintenance is not sufficient, damages cannot be repaired and their accumulation leads to deterioration of the organism, hence aging.

The mentioned evolutionary theories of aging assume that high levels of extrinsic mortality select for a reduced investment in physiological maintenance leading to a shorter life span (Medawar 1952; Williams 1957; Hughes and Reynolds 2005). Extended longevity on the other hand is associated with adaptations that reduce the risk of predation. Examples of that are subterranean living (Buffenstein 2005), the ability to fly (Austad and Fischer 1991), group or social living (Carey and Judge 2001; Keller and Jemielity 2006; Buffenstein 2005; Carey 2001) and intergenerational transfers (Amdam and Page 2005; Lee 2003). For eusocial insects it has been argued that differing levels of extrinsic mortality explain the different aging patterns between workers and queens (Heinze and Schrempf 2008; Parker 2011; Jemielity et al. 2005; Chapuisat and Keller 2002). In chapter I I discuss the implications of the evolutionary theories of aging and their application to eusocial insects.

In contrast to evolutionary theories, mechanistic theories of aging make predictions about the relationship of metabolic rate and life span. Larger organisms have a lower metabolic rate per unit of mass, a slower development and greater life span (Finch 1990; Collatz and Sohal 1986). For example, the life span of fire ant workers (*Solenopsis invicta*) is affected by both temperature and body size which both change the metabolic expenditure (Calabi and Porter 1989), but the relation between body size and life span may be positive or negative in the social insects (Parker 2011) as presented in chapter II.

Researchers are increasingly recognizing that social effects influence the evolution of aging. Newer approaches try to implement the social interaction between individuals to explain the observed aging patterns. One formal approach to explain post reproductive life span in humans shows that intergenerational transfers, which improve the survival of the grand-offspring, increase the selection pressure on survival at older ages leading to a longer life span (Lee 2003). Attempts to adopt this to eusocial insects where the effect of transferring resources leads to shorter life span of the helper / worker have not been formalized jet (Amdam and Page 2005), but a further approach is presented in chapter I, where the resource transfers within the colony are optimized to increase colony fitness.

Division of labor

All eusocial insects are defined by division of labor (Batra 1966; Wilson 1971), and division of labor is of importance when considering the life-histories of individuals within insect societies. The primary division of labor concerns reproduction and represents the fundamental novelty of the social insects which is comparable to the germ-soma differentiation in multicellular organisms (Boomsma 2009; Smith et al. 2008; Robinson 1992). The demographic outcome of this process is that, for workers reproduction and life span are negligible while the opposite is true for queens (Heinze and Schrempf 2008). Most intriguing is the fact that both queen and worker could have been reared from the same female larvae, thus they represent two different phenotypes of one genotype with distinct life histories affecting life span and reproduction (Munch et al. 2008). The Phenotypes are triggered by different gene expression patterns activated by different environmental condition throughout larval development (Evans and Wheeler 1999; Hoffman and Goodisman 2007; Winston 1991) and differing developmental times (Remolina and Hughes 2008) but ultimately result from division of labor.

Division of labor is an old and long used economic concept that can be traced back to Platos “The Republic” written in 380 BC. In 1776 Adam Smith mentioned, that division of labor leads to a qualitative increase in productivity (Smith 1776). He also distinguished between competitive and cooperative division of labor and in both cases, division of labor is proportional to the size of the marked (Smith 1776; Bell and Mooers 1997). Cooperative division of labor implies, that larger organization should posses a more diversified workforce to increase productivity because division of labor saves the loss of time involved in turning from one task to another and therefore increases the dexterity with which a repetitive operation can be performed (Smith 1776; Bell and Mooers 1997). In biological systems, evolution tends to produce more complex systems by gradually adding more levels to the hierarchy (Heylighen 2000). So-called evolutionary transitions appear in the integration of atoms into molecules, from molecules to cells, from single celled organisms to multicellular organisms, and during the transition from individual to group living. As for the economic division of labor, the individual subunits (molecules, cells, individuals) need to specialize to increase the group's fitness as compared to the individual fitness if not the group is selectively neutral (Michod et al. 2006; Michod 2006, 2007; Michod and Herron 2006). Consequently, one can only build a higher order system from simpler subunits after building blocks have evolved themselves via division of labor (Heylighen 2000). Therefore, larger biological systems comprise more and more diversified subunits (Bell and Mooers 1997; Changizi et al. 2002). The same process is visible in eusocial societies, where individuals are organized into reproductive units that themselves go through growth cycles and reproduce (Al-Khafaji et al. 2009). Different phenotypes arise to increase the fitness of the colony (Changizi et al. 2002).

In eusocial organisms the transition from simple or primitive to complex or advanced societies is associated with a loss of individual totipotency (Bourke 1999). This leads from a reduced reproductive potential of helpers or workers to complete worker sterility accompanied with increasing fertility and survival probability of the queen (Bourke 1999; Alexander et al. 1991). The increasing degree of caste specialization may either be behavioral or morphological and as in the economic examples, more complex and derived sociality as well as division of labor is phylogenetically correlated with larger colony size (Oster and Wilson 1978; Bourke 1999; Schmid-Hempel 1998; Hölldobler and Wilson 1990). In primitively eusocial species tasks are divided among subsets of behaviorally distinct colony members, while morphological differences between queens and workers are little (O'Riain et al. 2000; Lacey and Sherman 1991). Workers tend to be more generalized and perform different tasks, but reproductive individuals have an increased life span compared to the workers (e.g. Bombus bees, Polistes wasps, small ant colonies of *Rhytidoponera metallica*, *Platythyrea punctata)*(Hurd et al. 2007; Hartmann and Heinze 2003). In most eusocial colonies workers remain the physiological capability to reproduce but accomplish other tasks essential for the colony (Bourke 1988). Further division of labor only occurs after reproductive division of labor, which prevents individuals from pursuing their own egoistical interest, evolved (Simpson 2012; Korb and Heinze 2004). In complex advanced insect societies queen and worker differences appear early in larval development and lead to morphologically different phenotypes with distinct demographic properties (Smith et al. 2008).

Within the worker caste, further behavioral and morphological specialization may occur. Behavioral worker caste specialization is often age correlated leading to a temporal division of labor (age polyethism), whereat a series of tasks is accomplished with increasing age of the worker (Oster and Wilson 1978). Often young workers accomplish tasks within the colony, and as the worker age increases more risky tasks outside the colony are accomplished (Wilson 1971; Tofilski 2002; Odonnell and Jeanne 1995). In fact, the switch to outside activities such as foraging, that are associated with higher levels of extrinsic risk, has been shown to be the best factor to predict the life span of a honey bee (Rueppell et al. 2007a). In highly advanced eusocial species morphological distinct worker castes with specialized task repertoire occur that also show different aging patterns in response to the tasks carried out and independent from the body size (Chapuisat and Keller 2002; Hölldobler and Wilson 1990).

In insect societies, division of labor ultimately leads to the distribution of tasks to different specialized temporal or persistent castes and is therefore important to understand the diverging life-histories of individuals and task specific factors that affect the evolution of caste specific life span. I focused on these processes in chapter III.

Colony size

Life-history evolution has led to typical patterns of growth, reproduction and aging in animals (Stearns 1992). Colony size is a characteristic trait of social insects that is shaped by natural selection (Heinze 2008). Some eusocial species reach enormous colony sizes of several million individuals, while other species contain less than ten individuals at maturity (Beckers et al. 1989; Kaspari and Vargo 1995). Within species colony sizes vary less and those variations depend on life stage and the environment the colony is living in. Mature colony size is likely to be determined by a variety of ecological pressures (Bourke 1999), such as climate conditions (Kaspari and Vargo 1995) or the dependency on nesting cavities (Hansell 1987).

Colony size itself forms the basis for various life-history trade-offs and may be comparable to the effect body size has on organismal traits (Dornhaus et al. 2012; Bourke 1999). With increasing colony size, the collective organization is affected and several factors vary with colony size (Bourke 1999; Dornhaus et al. 2012; Oster and Wilson 1978). One of the factors is the reproductive potential of workers, which was shown to decrease with increasing colony size across different species (Bourke 1999). With decreasing reproductive potential the degree of dimorphism and division of labor rises and creates a positive feedback loop, that in turn promotes larger colony size (Bourke 1999). Thus, larger colony size is associated with more complex societies, defined by morphological differences between queens and worker, as well as increasing worker polymorphism, increasing nest complexity and communication systems including more complex modes of foraging (Bourke 1999; Michener 1974; Beckers et al. 1989).

The advantages of large colony size include better fighting abilities, the ability to overwhelm larger pray items, higher annual survival and an increased production of sexuals (Kaspari and Vargo 1995; Bourke 1999; Palmer 2004; Sorvari and Hakkarainen 2007). Larger colonies might have better abilities to buffer environmental constraints by keeping a larger reserve work force (Mirenda and Vinson 1981). Indeed, the number of inactive workers often increases with colony size (Robinson 1992; Dornhaus et al. 2009). In contrast to factors promoting large colonies those which limit colony size are less well studied. Colony growth can be restricted by limited availability of construction materials or nesting sites (Foitzik and Heinze 1998) and the foraging mode of a given species (Beckers et al. 1989) as evaluated in chapter IV.

Colony size also affects life span. Colonies with larger colony sizes show increasing life span and many species with small mature colony sizes are annual (e.g. Allopine bees, Bumble bees, some paper wasps and some sweat bees), whereupon larger colonies are often perennial (e.g. ants and honey bees) and may reach maturity after a long phase (up to 7 years) of colony growth (Tsuji 1996). This means, that especially monogyne species with large colony sizes must show long queen life spans to survive until the colony reaches a size where sexual offspring is produced. This might be the cause why queens of monogyne species live longer the those of polygyne species and why species with large colonies have queens with extraordinary life spans (Keller and Genoud 1997; Keller 1998; Hölldobler and Wilson 1990).

While queens of the many ant species ants become relatively larger in species with larger colonies (Bourke 1999), queen life span shows no association with colony size (Hölldobler and Wilson 1990). Worker life span instead tents to have a negative association with colony size when comparing related species (Schmid-Hempel 1998; Matsuura 1984; Rueppell et al. 2009). In chapter III I investigated the association of queen and worker life span with colony size. Even within one species colony size may affect life span. Honey bee (*Apis mellifera*) workers living in larger colonies live shorter than those living in small colonies (Rueppell et al. 2009). Therefore I tested the effect of the colony size worker were born in on life span in chapter II.

Goals of this thesis

In this thesis I studied the evolution of demographic properties in social insect societies. The main focus lies on the evolution of life span and colony size. Concerning life span, I focused on the factors that lead to the vast differences between queens and workers. Colony size represents one of these factors that affect life span but also has a number of other consequences for the insect society.

In chapter I I offer a novel approach on how worker life span patterns could have evolved in response to different levels of extrinsic mortality. While so far it has been argued that the evolutionary theories of aging explain those differences in life span, other authors did not include the hierarchical organization of eusocial insects and the shift in the unit of selection from the individual to the colony level into account. I lay out the evolutionary theories of aging and show their explanatory weaknesses if applied to a colony as the unit of selection. I then apply a trade-off model which includes the hierarchical structure to show how worker life span may have evolved. This approach covers intergenerational transfers within a colony which are proposed to have a strong effect on life span evolution (Lee 2003; Amdam and Page 2005). I further discuss empirical evidence on the resource allocation within insect societies to show, that the resource utilization and allocation within insect societies is under strong selection to increase colony efficiency and fitness.

For chapter II I conducted laboratory experiments to determine the life span of monomorphic *Lasius niger* workers and to disentangle the effect of natal colony and the effect of the social environment workers live in (Rueppell et al. 2009). The main question was if the life span of workers changes once the colony reaches the size where the foraging strategy changes from individual to collective foraging. To control for the effect the social environment has on life span, workers where housed in artificial colonies under similar conditions after they emerged from differently sizes colonies. The experiments showed that larger colonies produce larger workers that have significantly shorter life span then workers from smaller colonies.

Chapter III: After I found that, beside the social environment, the developmental stage or size of the colony influences worker life span I conducted a comparative study across eusocial Hymenoptera to study the effect of colony size on the life span of queens and workers. To this date no association of colony size and queen life span was found (Hölldobler and Wilson 1990), and for workers a negative association between worker life span and colony size was proposed (Schmid-Hempel 1998) or found within closely related wasp species (Matsuura 1991). For this study, I created the largest existing database on queen and worker life span of social Hymenoptera to test if colony size has an effect on queen or worker life span. Further I wanted to test if the increasing division of labor in larger colonies influences the divergence of queen and worker life span or if the increasing morphological differences (Changizi et al. 2002) in larger colonies explain the differences in life span.

Chapter IV: After showing that colony size has an effect on life span of both queens and workers, I conducted a study on the evolution of colony size. As shown in the introduction colony size has a number of social consequences (Bourke 1999; Dornhaus et al. 2012) and often larger colonies perform better in terms of colony defense, survival, sexual production and colony homeostasis (Kaspari and Vargo 1995; Bourke 1999; Palmer 2004; Sorvari and Hakkarainen 2007; Wilson 1974; Cole 1984).Therefore, the main question was: why do some species have very small adult colony sizes, when larger colony size is beneficial? I used per-capita productivity as a measure of colony fitness for eight species of cavity dwelling ants with small average colony sizes. It is believed that, these cavitiy dwelling species do not reach colony sizes where productivity is maximized as a result of disturbances (Kaspari and Byrne 1995). I further discuss factors intrinsic and extrinsic to the colony that may explain the average colony sizes of these species. In a further case study I tested the effect of colony splitting (polydomy) on productivity.

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Chapter I: Life span evolution in eusocial workers – a theoretical approach to understanding the effects of extrinsic mortality in a hierarchical system.

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Abstract

While the extraordinary life span of queens and division of labor in eusocial societies have been well studied, it is less clear which selective forces act on the short life span of workers. The disparity of life span between the queen and the workers is linked to a basic issue in sociobiology: How are the resources in a colony allocated between colony maintenance and reproduction? Resources for somatic maintenance of the colony can either be invested into quality or quantity of workers. Here, we present a theoretical optimization model that uses a hierarchical trade-off within insect colonies and extrinsic mortality to explain how different aging phenotypes could have evolved to keep resources secure in the colony. The model points to the significance of two factors. First, any investment that would generate a longer intrinsic life span for workers is lost if the individual dies from external causes while foraging. As a consequence, risky environments favor the evolution of workers with a shorter life span. Second, shorter-lived workers require less investment than long-lived ones, allowing the colony to allocate these resources to sexual reproduction or colony growth.

Keywords*:* *Worker, life span, social insects, extrinsic mortality, eusociality, trade-off*

Introduction

Life span is a highly variable trait. Across the tree of life, we find short-lived organisms like flies, which have a life span of less than a week. At the other extreme, long-lived species like elephants, tortoises or humans may live for more than 60 years (Carey and Judge 2000). Even within taxonomic groups, such as mammals, life span may vary by a factor of 60 (Carey and Judge 2000). Among insects, differences in the life span of solitary and social species reach a factor of 100 (Keller and Genoud 1997). This significant variation in life span is often explained by differences in the life histories of species. Moreover, life span may be highly variable even within a single species. Among eusocial insects, which are defined by a reproductive division of labor (Batra 1966; Wilson 1971), there is a distinct gap between the life span of reproductive queens and the mostly sterile workers (Carey 2001).

The observed disparity in the life span of queens and workers is determined by differential gene expression within the same genotype (Evans and Wheeler 1999; Hoffman and Goodisman 2007). From a diploid egg, worker or queen phenotypes can be reared with divergent demographic properties, such as life span and reproduction. The gap between worker and queen life span is most pronounced in species with caste dimorphism, among which the morphology of the queen and the worker castes differs. Honey bees and ants display a unique pattern of divergence in the life span of different phenotypes. Even among species with less pronounced morphological differences between reproductive and non-reproductive individuals, such as annual social wasps, life span differs depending on the task performed (Solis and Strassmann 1990).

The maximum life span of eusocial queens is 30 years (Keller and Genoud 1997; Hölldobler and Wilson 1990), while the average life span is considerably shorter due to high levels of mortality during colony foundation, especially in species with independent colony founding (Jerome et al. 1998). Workers may have a life span of less than a month (Rueppell et al. 2009; Hölldobler and Wilson 1990). An extreme degree of variation in life span within the same species is displayed by the invasive fire ant *Solenopsis invicta*. The queen outlives small workers by a factor of 30, even under protected conditions in the laboratory (Calabi and Porter 1989; Hölldobler and Wilson 1990).

In addition to this pronounced disparity in queen and worker life span, workers across different species also show a high degree of variation. The mean life span for eusocial hymenopteran workers ranges from 0.1 years for wasps to 1.6 years for ants (Keller and Genoud 1997; Schmid-Hempel 1998). Within the Fomicidea, the mean worker life span ranges from weeks to several years (Tohme and Tohme 1978; Carey 2001; Hölldobler and Wilson 1990). Even within a species, the life span of morphologically similar workers may vary. In the honey bee (*Apis melifera*), diutinus workers (winter workers) may outlive foraging and nursing workers by a factor of four, probably due to adaptations to temperate regions (Omholt and Amdam 2004), or to task-dependent life-history regulation (Rueppell et al. 2007a). In species with polymorphic worker castes, life span differs between different castes, either depending on size (Calabi and Porter 1989), or independent of size, but determined by the levels of extrinsic mortality associated with the tasks carried out (Chapuisat and Keller 2002). Compared with solitary insects, the life span of hymenopteran workers is prolonged. For example, the mean life span for solitary insects is 0.1±0.2 years (Keller and Genoud 1997), while ant, bee and wasp workers reach a mean of 0.9±1.1 years (Schmid-Hempel 1998). The life span of wasp and bee workers differs slightly from those of solitary insects but ant workers have much longer life spans. In the case of ants, the protected subterranean niche might also affect the evolution of life span.

Considerable work has been done to improve our understanding of the evolution of the extended life span and the high fertility of queens in eusocial species, especially in contrast to solitary insects (Carey 2001; Heinze and Schrempf 2008; Jemielity et al. 2005; Keller and Genoud 1997; Parker 2010). While researchers have focused on queen or colony life span, the evolution of different phenotypes, including the evolution of worker life span, has received less attention. The disparities in the life spans of queens and workers, or of different worker castes, have been explained by evolutionary theories of aging, and differences in extrinsic mortality depending on the task carried out (Chapuisat and Keller 2002; Rueppell et al. 2007a; Rueppell et al. 2007b; Rueppell et al. 2009). Recently, research on aging patterns in social groups has taken the effects of intergenerational transfers and relatedness on life span evolution into account (Lee 2003)(for a review see (Bourke 2007)). In this approach, the strength of selection on mortality, which is usually determined by the remaining fecundity, is additionally modified by transfer effects (Lee 2003). This approach can also be applied to social insects (Amdam and Page 2005; Rueppell et al. 2007a), and it could be used to explain the task-specific regulation of internal resources found in honey bees (Rueppell et al. 2007a; Rueppell et al. 2007b). It has been argued that the task-specific regulation of maintenance in honey bee workers could be important for preserving resources at the colony level (Rueppell et al. 2007b).

Here we want to test the effects of extrinsic mortality on optimal resource allocation to workers in order to explain the evolution of different aging phenotypes in a hierarchical trade-off setting that implements both the individual and the colony levels in eusocial species. First however, we will describe the important factors for the divergence in life span between queen and workers, and we will show how the evolutionary theories of aging, which connect the levels of extrinsic mortality and life span, may help to explain the evolution of worker life span.

Factors driving the differences in longevity between queen and workers

Two main factors, the division of labor and the level of extrinsic mortality, drive the extreme variation in life span within species of social insects. The two factors are interconnected, but in this chapter we want to point out the implications of each perspective. Later we show that the division of labor is the key to distributing the risk of extrinsic mortality among the different individuals in the colony.

Division of labor:

All eusocial insects are defined by division of labor. The primary division of labor concerns reproduction (Robinson 1992). In highly eusocial species, a single queen monopolizes reproduction (Hölldobler and Wilson 1977) while workers perform tasks related to colony growth and development (Robinson 1992). The queen lays all eggs which develop into workers, queens and males. Caste determination of diploid eggs, which develop into sterile workers or reproductive queens, is determined by environmental factors. Each individual develops into one caste, with distinguishable stage- and age-specific differences during its life cycle (Evans and Wheeler 1999; Hoffman and Goodisman 2007). In highly eusocial species (e.g., Atta leaf-cutting ants), worker castes are distinguished by behavioral and anatomical traits (Robinson 1992; Hölldobler and Wilson 1990). In addition, workers of the most highly eusocial species exhibit age polyethism, or a temporal division of labor (Jeanne 1986; Odonnell and Jeanne 1995a; Wilson 1971). Safe tasks are performed earlier in life, while risky tasks are delayed to higher ages (Tofilski 2002). The outcome of this process is that, for workers, reproduction and life span are negligible, while the opposite is true for queens (Heinze and Schrempf 2008). As evolution tends to produce more complex systems, the integration of individuals into colonies adds a new hierarchical layer during the evolutionary transition (Heylighen 2000). Generally, the division of labor in insect societies is comparable to the germ-soma differentiation in multicellular organisms (Boomsma 2009). The co-occurring specialization of individuals leads to a shift in the unit of selection from the individual level to the colony level. Concomitantly, with a shift in the unit of selection, different phenotypes for reproductive and somatic parts of the colony evolved. Consequently, a different role of selection on senescence should exist for the different castes of a colony.

Extrinsic mortality:

The level of extrinsic mortality of a colony member is directly correlated with its task and caste. The reduced life span of the workers relative to that of the queen in eusocial species has therefore been linked to the differing levels of extrinsic mortality (Parker 2010; Heinze and Schrempf 2008; Chapuisat and Keller 2002).

Workers perform all the risky duties within and outside of the colony. Tasks like foraging, nest guarding and defence entail a higher extrinsic risk of dying than the functions of a queen which resides in the center of the colony. From a colony perspective, the extrinsic risk is distributed to different individuals within the colony via the division of labor. Moreover, social insect workers can distribute the extrinsic risk to different ages in the life cycle by age polyethism (Tofilski 2002, 2006): this temporal division of labor improves the survival of the individual by shifting from the performance of safe tasks inside the colony early in life, to the performance of risky tasks like foraging later in life (Jeanne 1986; Odonnell and Jeanne 1995a; Wilson 1971).

Evolutionary theories of aging for social insects

To fully understand why workers die at younger ages than the queen, we need to address evolutionary theories of aging. According to these theories, aging evolves as a consequence of an age-related decrease in the force of selection (Medawar 1952), or by gaining early life benefits at the cost of late life disadvantages (Kirkwood 1977; Williams 1957). One derived prediction is that castes exposed to increased extrinsic mortality should show an increased rate of aging (Keller and Genoud 1997; Williams 1957). Empirical evidence supports this hypothesis (Stearns et al. 2000; Holmes and Austad 1994; Gorbunova et al. 2008; Shattuck and Williams 2010). However, recent theoretical studies on the link between extrinsic mortality and life span evolution suggest that more complex mechanisms may be involved than was previously thought (Moorad and Promislow 2010). For example, if density dependence acts mainly at older ages, or if survival is density-independent, the effects of extrinsic mortality on selection are reversed or even vanish (Moorad and Promislow 2010). It has been argued that differing levels of extrinsic mortality explain the distinct aging patterns of workers and queens in eusocial species (Chapuisat and Keller 2002; Heinze and Schrempf 2008).

Workers in many species, including the species in focus here, do not reproduce. Thus, it is hard to understand how deleterious mutations in the worker genome, being equal to the queen genome, can accumulate and be propagated in future generations. It has been stated that sterile workers are beyond the explanatory scope of these theories (Amdam and Page 2005; Amdam and Omholt 2002; Omholt and Amdam 2004).

Kirkwood’s disposable soma theory (Kirkwood 1977) explains how trade-offs between maintenance and reproduction lead to certain life histories, but it has the same difficulties in explaining the life histories of individuals within colonies, the unit of selection. When we look at the individual level, there appears to be no trade-off (Hartmann and Heinze 2003; Tsuji 1996): reproductive capacity and life span are low for workers, while the opposite is true for queens, which makes eusocial species a striking exception to the rule (Heinze and Schrempf 2008). It has also been argued that the fecundity / life span trade-off is reversed (Parker 2010), or that the variation within species goes against the normally observed life-history trade-off between reproduction and longevity (Heinze and Schrempf 2008). Individuals are embedded in colonies, and the exclusive consideration of those individuals can be misleading.

From a “superorganism” perspective (Wheeler 1911; Hölldobler and Wilson 2009), the unit of selection should be transferred to the colony level. Aging theories would then be able to explain the specific life histories of a colony, including life span, but do not account for the individuals within the colony: at the colony level, an investment in maintenance can be seen as an investment into workers that provide most of the functions needed for colony maintenance. Thus, it has been argued that workers are disposable at the colony level (Porter and Jorgensen 1981; Hölldobler and Wilson 2009), and that the disposable soma theory can be meaningfully applied to social insects to explain selection for resource investments in workers (Amdam and Omholt 2002). According to the disposable soma theory (Kirkwood 1977), long-lived colonies should invest more into somatic maintenance than short-lived ones. But a further trade-off appears: resources for somatic maintenance at the colony level can be invested in either quality or quantity, a trade-off that is comparable to the trade-off between quality and quantity of offspring (Roff 1992; Stearns 1992). Here we define quality as an investment in vitality, which defines the capacity of an organism to withstand destruction. It can be measured at any age by the chance of surviving to the next age (Strehler and Mildvan 1960; Gompertz 1825). An investment in quality is continuous, and can be independent of the size of an organism. High quality generates high investments in the physiological maintenance of workers, which would result in long-lived workers. Investing in quantity would help to build the workforce by adding more workers with lower quality. These two possible ways of investing in somatic maintenance at the colony level may be equally favorable. However, the trade-off between quality and quantity of maintenance is not implemented in the disposable soma theory. In order to understand how selection for different life spans in social insects may operate, it is necessary to reformulate our understanding of the mechanisms that shape trade-offs in hierarchical systems.

Different levels of extrinsic mortality lead to the differences in the life span of the queens and workers of a single species. But an evolutionary mechanism by which extrinsic mortality leads to different aging phenotypes in a colony setting has not been proposed.

The goal of this article is to propose a mechanism by which an optimal adjustment of worker life span/quality in response to extrinsic mortality can be selected for at the colony level. For this purpose, we will develop a hierarchical trade-off model incorporating the individual and the colony level. This model will then be tested by extending a model used for annual eusocial species (Macevicz and Oster 1976) with the hierarchical trade-off. While focusing on highly eusocial species with no conflict over reproduction, we will test the hypothesis that worker life span reduction may be adaptive at the colony level, and serves to reduce the loss of resources that could otherwise be invested in sexual reproduction. This would mean that the reduction of the life span of the worker relative to that of the queen may not be an outcome of weaker selection at older ages, as has been proposed in the evolutionary theories of aging, but may instead be a result of a quantity/quality trade-off that is actively selected for.

The hierarchical trade-off in eusocial species: a novel approach

Focusing on the trade-off between reproduction and maintenance (Gadgil and Bossert 1970), both hierarchical levels (individual and colony level) may have different implications.

1. At the individual level, investment in maintenance sustains bodily functions. This prevents organismal deterioration, and thus increases life span and future reproductive success. The level of investment in worker maintenance is low, which leads to a short life

span. Workers in many insect societies do not reproduce, or have a reduced ability to do so. Thus, there is no trade-off between maintenance and reproduction for workers. The queen distributes high levels of resources to maintenance and reproduction, which leads to a long life span and high fertility. Reproduction by the queen leads to the production of new individuals (workers, queens or males).

2. At the colony level (Fig. 1), sexual reproduction is accomplished by rearing queens and males. Male eggs may be produced by workers in several species (Bourke 1988). Virgin queens are produced from eggs laid by the queen under specific environmental conditions, which trigger gene expression that differs from the gene expression of workers (Evans and Wheeler 1999). Maintenance at the colony level includes investment in workers and results in building a workforce (colony growth) during the ergonomic phase of the colony. Alternatively replacing dead workers or changing the quality of the workers also represents maintenance on the colony level (see Fig. 1). These investments increase colony survival by enhancing its protection and increasing resource availability and generally help to buffer the environment (Schmid-Hempel et al. 1993; Kaspari and Vargo 1995; Franks and Partridge 1993). It should be noted, however, that having high levels of maintenance at the colony level does not necessarily mean that the investment per worker increases. Thus, having high levels of worker maintenance does not always produce the same outcome as having high levels of colony maintenance. Empirical data suggest that species with bigger colonies (higher investment in colony maintenance) have shorter-lived workers (low levels of investment in worker maintenance) (Schmid-Hempel 1998).

The resource flow of a colony (Fig. 1) is controlled by the influx and allocation within the hierarchical organization of the colony: resources are collected by foraging workers and brought into the colony. Resources that are not consumed by foraging workers are transferred to the colony to supply non-foraging workers, the brood and the queen. The economical use of resources for worker maintenance and the foregoing of worker reproduction increase the amount of resources available at the colony level. These resources can be channeled to either the maintenance or the reproduction of the colony. In short: if fewer resources are needed for maintaining individual workers, more resources are available for the reproduction-maintenance trade-off at the colony level (Fig. 1).

With this conceptual framework, it is possible to elaborate the benefits for the colony achieved by a reduction in investments in individual workers, or a shift from quality to quantity. Colony-level selection that acts to maximize colony fitness should shape investments in workers depending on the age independent mortality risk. These investments could be channeled in individual maintenance, thus achieving high levels of repair. Alternatively, changes in the initial investment, reflected in the body size of workers, could be modified. This can happen independently of worker quality. Each worker that dies of extrinsic causes means a loss of the resources already invested in the worker, as well as a loss of potential future work. An optimized adaptive demography could entail a lower investment in workers, which would reduce the individual life span, but would simultaneously reduce the potential loss of investments due to the high extrinsic mortality risks of the workers. The foraging worker needs to amortize the costs put into it from the colony.

Previous model specification

To show the effects of extrinsic mortality on the hierarchical trade-off in colonies, we modified an optimization model by Macevicz & Oster (1976) used for annual eusocial species. The original model offers a simple solution for incorporating individuals into a colony. The model runs for one season (200 days), producing a fitness value. This approach incorporates the effects of different allocation strategies into worker maintenance, but it avoids having to take into account the complicated allocation strategies over several seasons found in perennial species. The model assumes a bang-bang strategy: at the beginning of the season, all resources are invested in the production of workers (ergonomic phase). At the switching time (ts), all of the resources are invested in the production of sexuals. The model does not explore reproduction trade-offs between male or female reproductives; the sexuals produced represent queens, and will subsequently be called queens. The model by Oster & Wilson (1976), which was also used by Poitrineau et al. (2009), consists of two coupled differential equations:

$$\frac{dw(t)}{dt} = (1 - u(t))\gamma w(t) - \mu_w w(t) \qquad (1)$$

$$\frac{dq(t)}{dt} = u(t)\gamma w(t) - \mu_q q(t) \qquad (2)$$

Time (t) determines the number of workers (w) and the number of queens (q). The colony cycle starts in the spring with one inseminated and hibernated female that acts as a worker starting to forage (w (0)=1). All of the workers leave the colony to forage and return resources (γ) to the colony, which can then be allocated to colony growth and maintenance through the production of new workers or queens. The allocation function at the colony level (u) only take values of zero at the beginning of the season, meaning that all of the resources are invested in the production of workers (ergonomic phase); and one after the switching time (ts), which assumes that resources are exclusively invested in queen production at the end of the season ($t = 200$), an approach that has been criticized not to capture the biology of annual wasps (Poitrineau et al. 2009).This parameter represents a trade-off at the colony level, where resources can be allocated to either growth / maintenance or to sexual reproduction. This approach has been criticized for not capturing the biology of annual wasps (Greene 1984). Since the approach used here only asks about the effects of maintenance investments under different levels of extrinsic mortality, this simplification is valid, and it also avoids other assumptions about the timing of investments in sexual offspring or the workforce. The mortality of workers (μ_w) and queens (μ_q) is constant over time. Fitness is measured by the number of queens alive at the end of the season (see Poitrineau et al 2009 for results and discussion of the model).

To incorporate a density-dependent logistic growth for the colony, Poitrineau et al. (2009) added the following term (eq. 3), which leads to a reduction in foraging efficiency with increasing colony size, where ρ represents density dependency:

$$\gamma(t) = \gamma_0 - \rho w(t) \tag{3}$$

Our Extensions

The extensions to the previous model focus on the evolution of worker life span as an adaptive response to the age-independent mortality risk. Fig. 1 shows a schematic representation of the extended model. Colony fitness, measured as the number of queens produced at the end of the season, is the measure to be optimized. To account for the lower (individual) level trade-off, at which point the decision about the amount of resources that are used to maintain the workers is made, an allocation parameter (α) was added to the resource term:

$$\gamma(t) = (1-\alpha)(\gamma_0 - \rho w(t)) \tag{4}$$

This leads to a reduction in the resources brought back to the colony by each individual. The amount of resources invested in worker maintenance increases with α, generating a decrease in intrinsic mortality for the workers (μ_{wi}). Mortality is composed of an intrinsic mortality term and an extrinsic mortality term for both queens (μ_{qi}; μ_{qe}) and workers (μ_{wi}; μ_{we}). The extrinsic mortality for the queens is important because, once produced they leave the colony and need to survive until the end of the season to be included in the fitness measure. Extrinsic mortality of foraging workers and queens that left the colony are equal ($\mu_{qe}=\mu_{we}$).

$$\mu_w(t\,|\,\alpha)=\mu_{wi}(t\,|\,\alpha)+\mu_{we} \tag{5}$$

$$\mu_q=\mu_{qi}+\mu_{qe} \tag{6}$$

The intrinsic mortality of the queens (μ_{qi}) is fixed, and it is also the lowest value that worker mortality (μ_{wi}) can reach, since queens usually represent the phenotype with the longest life span. Since the costs of worker maintenance need to be linked to the reduction in daily foraged resources, the following equation was included in the model. It assumes that reaching the same low mortality as the queen would consume all of the resources gained while foraging. Also included is the reduction in foraging returns caused by the density dependency (Eq. 3), which leads to lower investments in workers when the foraging returns decline. The queens that have been reared to maturity leave the colony, thus they do not take resources from the colony for maintenance.

$$\mu_{wi}(t\,|\,\alpha)=\frac{\mu_{qi}}{\alpha(\gamma_0-\rho w(t))/\gamma_0} \tag{7}$$

It should be noted that, in the extreme case in which $\alpha=1$ (i.e., all foraged resources are kept by the foraging individual) and w(t)=0 (i.e., there is no reduction in foraging efficiency), the intrinsic mortality for workers is as low as it is for the queen. This would lead to a solitary life for each individual, because no resources would be transmitted to the colony. With reduced investment in workers, the mortality increases, but the resources of the colony also grow.

With the changes mentioned in (3-7), the model equations become:

$$\frac{dw(t)}{dt}=[1-u(t)]\gamma(t)w(t)-\mu_w(t\,|\,\alpha)w(t) \tag{8}$$

$$\frac{dq(t)}{dt} = u(t)\gamma(t)w(t) - \mu_q q(t) \qquad (9)$$

The number of queens at the end of the season (t=200 days) is the fitness value, which is optimized by changing the switching time (*ts*) and worker maintenance (α) (Fig. 1). The optimization was done using the optim function with the L-BFGS-B method from the R stats package (R-Core-Development-Team 2010).

Results

The results of the model show that the negative effects of increasing extrinsic mortality on colony fitness (Fig. 2D) can be attenuated by adjusting the quality of workers (Fig. 2A) via an evolutionary process. This indicates that the change in worker maintenance is adaptive at the colony level because it is able to buffer the reduction in colony fitness deployed by increasing levels of extrinsic mortality. Changes in the life expectancy of workers are driven by density dependency and extrinsic mortality, but are attenuated due to reduced maintenance investment in higher extrinsic mortality settings. Fig. 2 shows the results of the optimization model (parameters: γ=0.15, μ_{qi}=0.005, ρ=0.0024). For 100 levels of extrinsic mortality (μ_{we}=0-0.06), the optim function is used to find optimal values for maintenance (α) and switching time (ts) that maximize the number of queens alive at the end of the season.

Investment into workers:

Fig. 2A shows that optimal investment in workers (α) decreases with increasing extrinsic mortality risk. This reduced investment in worker maintenance increases the intrinsic mortality of workers and decreases life expectancy (Fig. 2E/2F). On the other hand, changing maintenance investment reduces the loss of resources for the colony due to the death of workers, because the daily need for resources by the workers decreases. The reduced investment distributes the resources that the colony gains through foraging to more individuals, which in turn die earlier. The dashed line indicates the fixed α -value used to show the divergence in colony fitness (Fig. 2D) and worker mortality (Fig. 2C).

Worker mortality:

Fig. 2C shows the increase of total worker mortality with increasing extrinsic mortality. Total worker mortality is composed of intrinsic and extrinsic mortality ($\mu_{wi} + \mu_{we}$). The reduction in the investment into worker maintenance increases worker mortality (μ_{wi}), in addition to the rise in extrinsic mortality (μ_{we}). The rise in extrinsic mortality alone is indicated by the dashed line. The reduced investment in workers under higher levels of extrinsic mortality keeps the net energetic efficiency of a worker from becoming negative, but it is still decreasing (not shown).

Sexual reproduction (Fig. 2D):

With increasing extrinsic mortality, the number of queens alive at the end of the season decreases. The dashed line indicates the queens produced if there were no change in investment (α) or switching time (ts) with increasing risk, while the solid line shows the results from the optimization. The difference between the two lines represents the fitness benefits of changing strategies with increasing extrinsic risk. It shows that a reduced or adapted investment into workers and a change in switching time are more efficient than keeping the strategy that is favorable in low-risk environments. Due to the interaction of α and ts, we also run the model with either α or ts values obtained from the optimization, while the other parameter (α or ts) obtained in the low extrinsic mortality setting was held constant. The effect of ts on sexual reproduction is stronger than the effect of α, but the effect of α increases with increasing extrinsic mortality explaining up to 44% of the fitness differences. Consequently, if colonies are able to adapt investment into workers to extrinsic risk, they are capable of surviving at higher levels of extrinsic risk.

Switching time:

The optimal switching time to sexual reproduction is earliest in the low risk environment, and it increases with increased levels of mortality (fig. 2B). In low risk environments, the switching time is driven by density dependent effects: low extrinsic mortality increases the number of workers. This leads to a decrease in the resources brought back by each individual, making it efficient to produce queens early. Additionally, queens have a low intrinsic risk of dying (μ_{qi}=0.005), which favors their early production. Queens increase the fitness of the colony only if they survive until the end of the season. In high risk settings, queens produced early cannot survive until the end of the season, and the density-dependent effects do not reduce the foraging efficiency of workers. As a consequence, the colony switches later to the production of queens.

Worker life expectancy:

Fig. 2E shows worker life expectancy at the beginning of the season under different levels of extrinsic mortality, while Fig. 2F represents the life expectancy at switching time (*ts*), when the worker population reaches its maximum. The dashed line indicates worker life expectancy without an adjustment to increasing levels of extrinsic mortality. The solid line represents the life expectancy using the optimal alpha value (α). The divergence between the dashed and the solid line represents the changes in life expectancy due to changes in maintenance investments. At the beginning of the season (Fig. 3E), the life expectancy of workers is ~74 days (calculated by (-1/log(survival))) with low extrinsic mortality, and it reaches ~11 days under the highest level of extrinsic mortality (μ_{we}=0.06). The difference in life span due to changes in investment reaches ~5 days. At switching time (Fig. 2F), life expectancy is ~29 days and it declines to ~11 days at high levels of extrinsic mortality. The difference between Fig. 2E and Fig. 2F is driven by density dependency, which also reduces the amount of resources available for worker maintenance. Reducing the effects of density dependency yields the same results for worker life expectancy, but leads to the production of higher numbers of workers and queens. The changes in life expectancy due to changes in maintenance investments are minor compared to the effects of density dependency and extrinsic mortality, but they nonetheless lead to major changes in colony fitness (Fig. 2D)

For a comparison of the model we used parameters from Poitrineau et al. (2009). Their parameters (productivity γ=0.035, mortality of queens μ_q=0.01, size dependency factor ρ =0.005) yield the result that no queens are produced until the end of the season (result not shown). The foundress acting as a forager dies without the production of new workers. Since productivity now incorporates maintenance costs, which were not included in the earlier models, productivity needs to be raised. We have also reduced the intrinsic mortality of the queen because we added extrinsic mortality separately, which leads to lower, equal and higher mortality, as used in (Poitrineau et al. 2009).

High levels of density dependency (ρ>0.05) have the opposite effect (not shown) on the investment into workers. Low levels of extrinsic risk lead to high worker survival, and the individual foraging returns decline due to density dependence. The optimal strategy is then to invest less into workers and increase their mortality in the low-risk environment to overcome the effects of density dependence. Lower levels of density dependency (ρ<0.002) lead to unreasonable large colony sizes and high numbers of sexuals produced at the end of the season (>500). The reduction of worker maintenance with increasing extrinsic mortality follows the same trajectory, but is less pronounced.

Discussion

The presented optimization model shows that a reduction of worker life span may be an adaptive response of the colony under the influence of extrinsic mortality. The fitness of the colony improves by a risk dependent investment into its workers. This indicates that the investment into workers should be under strong selection. The hierarchical trade-off within a "superorganism" can explain how extrinsic risk alters resource flows within colonies. Additionally, the integration of individual-level trade-offs within colony-level trade-offs explains why workers seem to have "reversed" trade-offs. Under increasing levels of extrinsic mortality, this leads to a contradictory process in which a shorter worker life span, as a result of economic resource management, leads to a higher fitness for the colony. This occurs by reducing the risk of losing investments made into individuals and simultaneously offers the option to invest the saved goods into building a stronger workforce or to produce more sexual offspring. The regulation of worker life span may have evolved as an energy-saving mechanism at the colony level (Amdam and Page 2005; Amdam and Omholt 2002).

Even though the presented model represents the biology of an annual species following a bang-bang strategy, we assume that the economical considerations underlying the hierarchical trade-off presented here apply to all social insects.

The hierarchical trade-off shown here can be used to explain why (via environmental selective pressure) the colony is capable of protecting resources by modifying the life span of workers internally. The model has implications for the investment into worker quality under different levels of extrinsic mortality. The results show that under certain conditions it is useful for the colony to be parsimonious with its resource investments into individual workers. The lower the costs of a worker in an environment with a high age independent mortality risk, the higher the chances that the worker will be capable of amortizing its own production costs. At lower levels of extrinsic mortality higher levels of maintenance investments into workers are favorable. Colonies would not survive without adapting the maintenance investment to the different levels of extrinsic risk because the number of queens produced at the end of the season could drop below one individual.

High risk environments would have a more negative effect on colony fitness if the extrinsic risk faced by individuals, could not be distributed to different castes. This result is consistent with Michod’s (2006) finding that the conversion from cell groups to multicellular organisms implies a shift in the level of selection to the colony, as a specialization of reproductive and vegetative functions is needed. Maintenance investments are channeled into the somatic maintenance machinery of the colony, instead of into the individual worker (Amdam and Omholt 2002; Amdam and Page 2005). Resources for somatic maintenance at the colony level could be invested into the quality or the quantity of the workers. Since individuals are loosely integrated and do not gain value throughout their life time (no learning), they are easy to replace, given that production costs are low. Most social insects form spontaneous task groups instead of persistent groups (Anderson and Franks 2001), but a task specialization of individuals is possible (Chittka and Muller 2009). This organization ensures a high degree of flexibility, while also allowing for an exchange of individuals without the attenuation of individual efficiency. High levels of individual flexibility are a result of low integration. This effect can be seen in simple multicellular organisms (Galliot and Schmid 2002) with loosely integrated cells and a high level of regenerative ability. If groups (of cells or workers) were to persist over time and increase in efficiency, an exchange of individuals could lead to a decrease in group efficiency, making it important to maintain the members of the group.

The model developed here shows clearly that the colony should keep the loss of resources low by economizing the investment in individual workers. With the proposed mechanism, extrinsic mortality acts directly on worker life span evolution.

As a proximate mechanism for the regulation of aging in honey bees, the lipoprotein vitellogenin has been proposed (Amdam and Omholt 2002). It has been shown that the regulation of aging via vitellogenin also controls the depletion of nutrients in honey bees (Amdam and Omholt 2002). Under high levels of extrinsic mortality, a colony with a mutation leading to a reduced investment in workers through a change in the regulation of vitellogenin could outcompete a colony without this mutation, because more resources would be available. This trait would be directly selected for, and could become fixed in a population facing high levels of extrinsic risk. In a mutation accumulation framework, the force of selection declines with age, and deleterious mutations leading to a reduced life span would not be selected against. This is the common explanation for the link between extrinsic mortality and life span. We argue that the mechanism proposed here is a much simpler evolutionary process by which worker life span could be shaped. The reduction of worker life span would be adaptive at the colony level, since it increases the fitness of the colony. Additionally, the model presented combines energetic benefits and the effects of extrinsic mortality to an entity that can be optimized by natural selection.

Empirical evidence

The model shows that a reduced investment in workers with increasing extrinsic mortality is economical for the colony. But the model fails to answer the question of which physiological adaptations would lead to a reduction of resource loss due to the extrinsic death of workers. Here we will discuss several adaptations in colonies of social insects that minimize resource loss caused by age-independent mortality. Generally, the model presented needs data on energy costs for workers depending on caste, task and extrinsic hazard, as well as data on longevity and maintenance costs. Currently, however, the data records in the literature are very weak. Nevertheless, there are some examples that might lead to future research.

One possible approach is to compare physiological adaptations of closely related species: *Lasius niger* and *L. flavus* are closely related, but workers experience different levels of extrinsic mortality. *L. niger* is a synanthropic species with a broad food spectrum (Seifert 2007). This leads to higher extrinsic mortality during foraging. *L. flavus* has a mainly subterranean way of life, including trophobiosis with subterranean aphids (Seifert 2007). This leads to a lower extrinsic risk for foragers. Following from the model, *L. niger* should invest less in individual workers than *L. flavus.* A comparison of worker life span data reveals that *L. niger* workers live one to two years (Parker et al. 2004), whereas *L. flavus* workers may live up to 10 years (Schreiber 1969). A comparison of biomass reveals that *L. niger* workers are lighter (0.58 mg dry weight) than workers of *L. flavus* (0.86 mg dry weight) (Brian 1978). Following the predictions from our model, the species with the higher mortality risk reduces the initial investment/production costs for workers relative to the species with the lower risk. Thus, losing one worker to an extrinsic risk is not as costly for *L. niger* as for *L. flavus*. Additionally, metabolic costs represent a measure of daily costs for the individual, including maintenance/repair, which determine the quality of the individual. The smaller workers of *L. niger* show lower respiration rates per mg of biomass (1.08 mm^3 O_2 mg^{-1} h^{-1}) than *L. flavus* (2.04 mm^3 O_2 mg^{-1} h^{-1}) (Brian 1978). For an interspecies comparison, the difference in the metabolic rates of *L. niger* and *L. flavus* can be interpreted as higher maintenance costs for the species with the lower extrinsic mortality as predicted by the model.

The fire ant *Solenopsis invicta* has a polymorphic worker caste. The head width of large workers is twice that of small workers. Large workers live 50% longer than minors in treatments with 24°C. Indeed, their maintenance costs measured as respiration rate per mg tissue at 24°C of 0.9 µl O_2 h^{-1} mg^{-1} are lower than those of smaller workers 1.55 µl O_2 h^{-1} mg^{-1} (Calabi and Porter 1989), but the absolute economic costs per individual are, due to their greater size, equal to those of at least four small workers (Calabi and Porter 1989). The large workers only forage for the last 25% of their lives, whereas small and medium-sized individuals do so for about 50% at the end of their lives (Calabi and Porter 1989). This again shows that the productivity/cost ratio for the worker is under strong selection. This process keeps colony efficiency at high levels. The life histories of individuals within the colonies are adjusted to keep resources within the colony. In this case, the timing of foraging in the life cycle is more limited to later ages among the larger, more expensive workers than among smaller workers, which reduces the potential loss generated by extrinsic mortality.

In *Solenopsis invicta* and other species, the first workers produced at the start of colony development tend to be smaller (cheaper) and shorter lived (5%) than the individuals produced later in the colony development (Oster and Wilson 1978; Porter and Tschinkel 1985). In species with single founding queens, this leads to a higher number of workers that can be reared from the limited resources. This adaptive process at the colony level maximizes early colony productivity, while increasing individual efficiency via the parallelization of tasks, and spreading the risk of forager mortality (Porter and Tschinkel 1986). In addition, these early workers develop faster, which is also important at the early stages of the colony (Porter 1988). These findings suggest that there is a quality-quantity trade-off within the maintenance investments at the colony level, and that it is selected for in order to increase the fitness of the colony.

Several studies have also shown that the quality of the workers declines when they perform more dangerous tasks. In species with age polyethism individual nutrient stores are depleted during more risky tasks to keep resources within the protected colony and to reduce the chances of losing those resources due to the death of foragers (Amdam et al. 2004; Odonnell and Jeanne 1995b; Amdam and Omholt 2002).This process includes a reduction in the fat body, water content, as well as in immune cell count (Amdam et al. 2004). Thus, the onset of foraging and not age is the best predictor for life span in the honey bee (Rueppell et al. 2007).

If the immune response is triggered (without an actual infection) in several individuals of a single bumblebee colony, the production of sexuals is highly reduced, without individuals getting sick (Moret and Schmid-Hempel 2000). This shows that the somatic maintenance of workers is turned down to a level that keeps the ratio of productivity to costs high. From a colony perspective, it means that resources are kept within the protected colony to avoid loss. But more costly mechanisms, such as the immune system, can still be activated if needed.

The weaver ant *Oecophylla smaragdina* shows a bimodal size distribution, which is correlated with a pronounced division of labor (Chapuisat and Keller 2002; Hölldobler and Wilson 1990). Minor workers stay within the nest, while major workers attend to more risky tasks. In laboratory experiments with a low level of extrinsic risk, minor workers show a significantly higher survival probability than the major workers, even though the majors have three times the body mass (Chapuisat and Keller 2002). This shows that the level of extrinsic mortality may be more efficient in shaping survival than body size and metabolism in weaver ants, and that life span may be less affected by physiological constraints (Chapuisat and Keller 2002). The quality (expressed as life span) of the workers seems to be independent of size. Generally, different morphological worker castes tend to accomplish specialized tasks more efficiently (Wilson 1983). If the specific task or tasks of a morphological caste are linked to other levels of extrinsic mortality, the investment in quality seems to be selected accordingly. This is in line with the framework of the hierarchical trade-off presented here.

Our simple model is just a first step to understand trade-offs in hierarchical systems. Even if the model represents a special case within the social insects (annual eusocial species and the assumption of a bang-bang strategy instead of a graded transition), we are sure the economical consideration within can be expanded to perennial species, as our empirical evidence section shows. A more general model incorporating a wider range of the variety of lifestyles found within the social insect would be a further step to understand the implications of the presented process but so far the data on maintenance cost and quality quantity trade-offs of social insect worker are not available und thus we decided to start with a simple but several times evaluated model. The general nature of the model reduces the assumptions that need to be made. At the same time this offers the opportunity to extend the specification of the model. The model predicts the optimal investments in workers but does not cover for example life span evolution of queens or the case of multiple worker castes. Moreover, including the production costs for different worker castes/sizes and adding age-dependent mortality could help us to better understand how colonies are able to distribute extrinsic risk among their members. This approach could also be extended to cover worker reproduction and its effects on colony fitness (costs for ovary development, conflict over reproduction). However, in spite of these limitations, this model shows how extrinsic mortality might feed back to the colony, and how selection could work to increase colony fitness under certain conditions.

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Figures

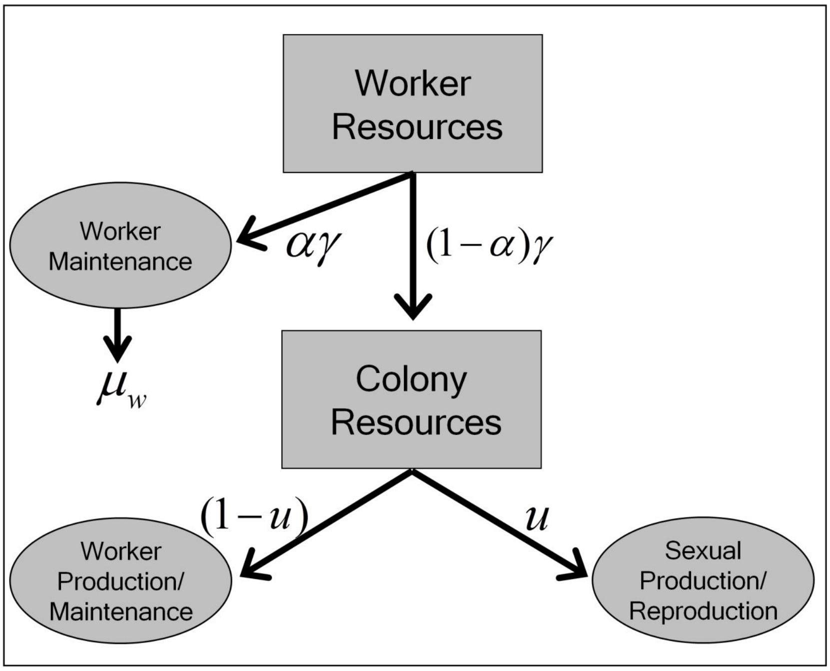

Fig. 1: Hierarchical trade-off model for eusocial species.

Simplified hierarchical trade-off with a focus on workers for eusocial species, including two trade-offs at the colony and the individual levels. Arrows indicate resource flows. Resources are obtained by workers that do not reproduce and are allocated toward worker maintenance (α) and/or the colony (1-α). At the colony level, resources that are not consumed by workers can be allocated to sexual reproduction (u) and/or maintenance (1-u), such as the production of new workers or different levels of worker quality.

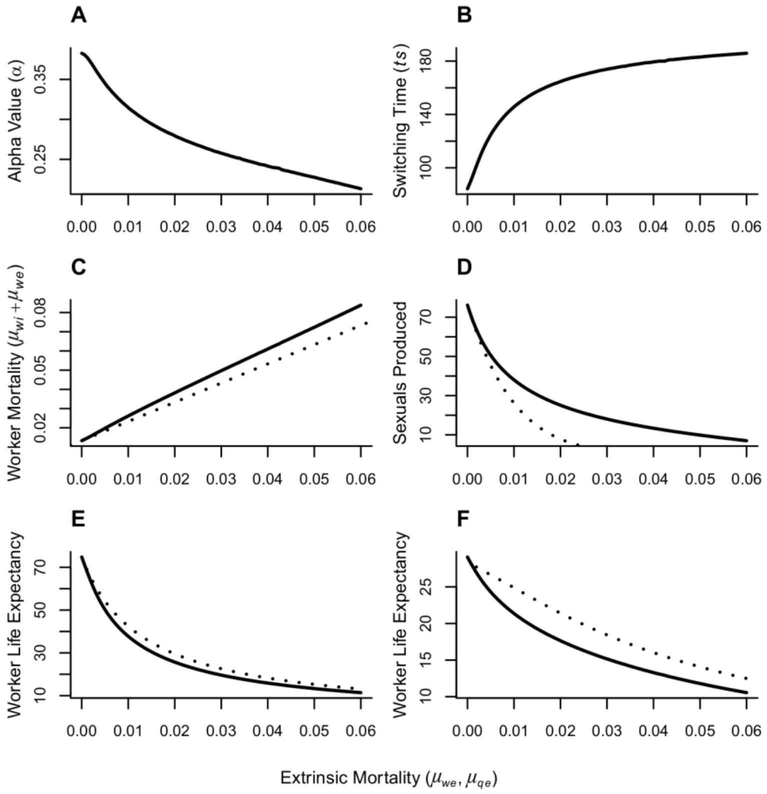

Figure 2: Model results under different levels of extrinsic mortality.

The horizontal axis represents the values of extrinsic mortality used to run the model(μ_{qe} ; μ_{we}). A) and B) show the optimized parameters (α , ts ,) from our model. In C)-F) the solid lines indicate the results using the optimal values of α and ts , The dashed lines indicate results if the colony did not change the maintenance investments (α) or the switching time (ts) with increasing extrinsic mortality (α =0.38 ts =84). A) Optimal investment into workers (α) decreases with increasing extrinsic risk. B) Denotes the switching time (ts), where the colony switches to the production of sexuals. C) The number of sexuals alive at the end of the season (maximized by finding optimal values for switching time (ts) and maintenance investments into workers (α)) decreases with increasing extrinsic mortality. D) Worker mortality combines intrinsic and extrinsic mortality ($\mu_{wi} + \mu_{we}$). The dashed line denotes the increase of extrinsic mortality. The difference between the dashed and solid lines shows the effect of the changing investment in worker maintenance. E) Worker life expectancy at the beginning of the season with different levels of extrinsic mortality. F) Worker life expectancy at switching time. At switching time, the worker population reaches its maximum. The difference in worker life span between E) and F) is due to the reduction of foraged resources caused by density dependency. Used parameters: γ=0.15, μ_{qi}=0.005, ρ =0.0024, μ_{qe}= μ_{we} =0-0.06.

Chapter II: Worker quality is determined by colony stage and not social environment in *Lasius niger*

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Abstract

Social insects are well known for the divergence in life span between short-lived, non-reproductive workers, and long-lived reproductive queens. The life span of queens of many species of perennial insects, in particular termites and ants, may be up to 3 orders of magnitude higher than conspecific workers. A promising approach to elucidate the ultimate and proximate factors that determine life span has been suggested by research on honey-bees, where worker life span depends on the seasonal state of the colony, as well as on the colony size. Here, we studied the mortality of artificial cohorts of *Lasius niger* workers that emerged from different colony stages. Early stage colonies have just been established, are small in number, produce workers of small body sizes and show individual foraging behavior. In contrast, late stage colonies are larger and consist of workers with larger body size that forage cooperatively. In our experiment, workers from both colony stages were kept under similar social environment (colony size). We found that body size and life span depended on the stage of the colony the workers originated from. Workers that had emerged from small colonies (18±8 workers) were 6 % smaller than those from larger (250±79 workers) colonies but had a 49% increase in survival. Social environment measured as experienced cohort size affected the rate of mortality, which increased as the cohorts became smaller with time. This detrimental effect of shrinking cohorts was independent of worker age and it was also independent of worker origin. Workers from early colonies were similarly affected by shrinking cohorts as were workers from late stage colonies. In conclusion, we show that the stage of the colony the workers were reared in has a stronger effect on worker life span than the experienced social environment and suggest that a trade-off between growth and life span of *L. niger* workers during colony ontogeny would function as a proximate mechanism.

Keywords: *Social insects, life span, survival, Lasius niger, worker, colony ontogeny*

Introduction

Social insects offer the unique opportunity to study the evolution of life span and aging because they show striking differences in life span between queens and workers as well as for different worker castes (Keller and Genoud 1997; Heinze and Schrempf 2008; Jemielity et al. 2005; Calabi and Porter 1989; Chapuisat and Keller 2002). In social insect colonies, individuals cooperate and form colonies which constitute a higher level of biological organization (Hölldobler and Wilson 2009) leading to a life span differential between workers and queens (Kramer and Schaible 2013). These differences in demography among workers and queens have a profound effect not only on colony fitness but also on colony demographic rates, such as colony growth rate, colony size and colony longevity (Odonnell and Jeanne 1995a; Billick 2003; Hölldobler and Wilson 1990).

To understand which factors and trade-offs shape the demographic rates within a species, it is a useful strategy to describe how these rates are affected by environmental states or by the state of the colony (Blondel et al. 1992; Rueppell et al. 2009).

Factors affecting life span in social insect workers

The evolution of the extreme differences in life span within colonies of eusocial insects have been examined against the background of evolutionary theories of aging. Different life spans evolve as a response to differences in extrinsic mortality faced by queens and workers (Keller and Genoud 1997; Heinze and Schrempf 2008; Parker 2011). In particular, extrinsic mortality has been thought to play a major role driving the evolution of worker life span, in fact different levels of extrinsic mortality may even explain life span differences between worker castes of *Oecophylla smaragdina* (Chapuisat and Keller 2002). Apart from extrinsic mortality, several other factors, such as body size of workers, have been shown to influence worker life span. For example, larger workers of *Solenopsis invicta* show an increased life span as compared to smaller workers which also have a higher metabolic rate (Calabi and Porter 1989). A negative correlation of life span and body size was found for *O. smaragdina* and *Acromyrmex subterraneus* (Camargo et al. 2007; Chapuisat and Keller 2002). Apart from body size, worker life span can also be affected by diet. The diet throughout larval development is a determinant of honey bee (*Apis mellifera*) worker life span (Maurizio 1950), and worker life span in *L. niger* is influenced by the balance of protein and carbohydrate in the diet of adults (Dussutour and Simpson 2012).

An important behavioral component affecting the life span of workers in a colony is age polyethism, the temporal division of labor. Workers in many species switch from safe tasks within the colony early in life to risky tasks like foraging later in life (Odonnell and Jeanne 1995a, 1995b; Tofilski 2002; Camargo et al. 2007). in fact, for honey bees it has been shown that the onset of foraging outside the hive is more important than chronological age for honey bee aging (Rueppell et al. 2007).

Furthermore, intrinsic aging rates can be altered by social environment (Parker 2011), such as the colony state, which can directly influence the temporal division of labor. Starving colonies may recruit young nursing workers to attain foraging early in life and by that reduce their life span, a potentially reversible process that has been linked to vitellogenin, juvenile hormone and insulin pathways (Munch et al. 2008; Rueppell et al. 2007; Woyciechowski and Moron 2009; Robinson 1992; Huang and Robinson 1996; Amdam and Omholt 2002). In addition, the seasonal state of honey bee colonies has been shown to affect worker life span. Workers born in summer live for an average of 15-38 days, while workers born during winter can survive for 140 days and longer during (Ribbands, 1953; Fukuda and Sekiguchi 1966, Winston et al 1981, 1983). Also colony size may affect worker life span. Workers in large honey bee colonies live shorter than worker in small colonies (Rueppell et al. 2009), and winter survival of *Formica neorufibarbis* workers decreases in larger colonies (Billick 2001).

In conclusion, several factors connected to colony state have been identified to modify the aging patterns of workers.

Lasius niger as a model organism

The ant species *L. niger* is known for having the longest lived queens with a maximum life span of 28 years, while workers have a life span of 1-2 years (Hölldobler and Wilson 1990). In species, such as *L. niger*, with claustral, independent nest foundation (Keller and Passera 1989) queens do not forage but raise the first workers solely on their body reserves and sacrifice worker size for worker number (Tschinkel 1988). These first workers, often called "minims" or "nanitic workers", have been shown to be more efficient at brood rearing (Porter and Tschinkel 1986), are smaller than workers produced in more mature colonies and in *Solenopsis invicta* their life span is 5% shorter than that of regular sized workers (Porter and Tschinkel 1985, 1986; Tschinkel 1999; Wilson 1983). In *L. niger* the switch from the production of small to regular sized workers is accompanied by a switch to cooperative foraging that is displayed by colonies larger than 75 individuals (Mailleux et al. 2003). This switch to cooperative foraging represents a change in the state of the colony which depends on colony size rather than age (Mailleux et al. 2003).

Aim of the study

In this study we tested whether the state of the colony or the social environment determines the quality and consequently the life span of workers in a eusocial insect. We expect diverging life span patterns for workers from small and workers from large colonies, as they are reared in colonies at different behavioral stages (Mailleux et al. 2003). We decided to use *L. niger* as a model organism, because this species shows a behavioral shift in foraging strategy at a colony size of ~75 individuals which represent a change in the stage of the colony and is accompanied by the production of regular sized worker that are more efficient in retrieving food during foraging trips (Mailleux et al. 2003). Our assay consisted of two batches of ants that were composed of workers that were obtained from differently sized colonies (18±8 and 250±79 workers). Workers from each batch were transferred into artificial colonies with a similar colony size to control for the effect of the social environment.

Methods

Ant collection

17 dealate (wings are shed after the mating flight) *Lasius niger* queens were collected on the 13.7.2009 after a mating flight in Rostock, Germany (Lat=+54° 5' 35.86", Lon=+12° 6' 40.50"). After collection from the wild, the queens were housed in test tubes containing water tanks in a climate chamber under constant temperatures (22°C) without light. After emergence of the first brood the test tubes were opened and placed in plastic boxes (12x17x4cm). Food (honey-water and boiled, chopped house crickets, *Acheta domestica*) was provided and replaced weekly and the water tanks were renewed as needed. Throughout the winter (15.10.-15.3.) the plastic boxes containing the colonies were housed in a cooling box and placed in a dark room at ambient temperatures.

Experimental setup

We created artificial colonies of individuals of approximately the same age. For that purpose we checked colonies weekly and removed newly hatched callow workers from all our colonies once per week throughout June and July 2010 and 2011. These workers were then merged into artificial colonies of same-aged workers (with a maximum age difference of 3-4 days due to the time needed for cuticular hardening). We will henceforth refer to these artificial colonies as cohorts. Eggs and larvae from the source colonies were provided, but no queen was introduced. The cohorts were kept at constant laboratory conditions at 22°C, as temperature has a strong effect on worker life span (Calabi and Porter 1989). Due to the fact that individually distinct cuticular hydrocarbons develop after emergence (vander Meer et al. 1989), we did not observe any aggressive interaction or an aggregation of workers that could have emerged from the same colony.

In addition to the weekly feeding regime, we checked for dead workers and supplied the cohorts with brood from the natal colonies so that the workers could engage in natural colony tasks, and removed pupae to avoid additional workers in our cohorts. Dead workers from each experiment were collected and pooled for head width measurements.

For the first experiment (Exp1) we used cohorts obtained after the first hibernation of the natal colonies. At the beginning of June 2010 natal colonies contained on average 18±8 workers (range: 8-45). We established 6 cohorts including overall 217 workers (mean cohort size: 36±7, range: 28-43) by collecting one cohort per week between June and July 2010.

For the second experiment (Exp2) we repeated the same procedure as for Exp1 but after the second hibernation of the natal colonies in 2011. At the beginning of June 2011 colonies contained on average 250±79 workers (range: 122-392), thus well above the threshold to cooperative foraging and the production of regularly sized workers (Mailleux et al. 2003). We established 3 cohorts including a total of 173 workers (mean cohort size: 58±18, range: 42-73). Our experimental cohorts did not differ significantly in cohort size (t-test: $p=0.190$, $df=2.23$, $t=-1.86$), and all cohorts were smaller than the critical size of 75 individual that has been shown to induce behavioral changes of the colony (Mailleux et al. 2003). Not all workers from both experiments were dead by the end of the study. These animals entered the analysis as right censored. In January 2013, 6.5% (Exp1) and 17.9% (Exp2) of the workers were still alive.

Head width measurement

To account for different levels of investments into individuals from the different experiments, dead workers of the different experiments were collected and pooled. Head width was measured in pixels from pictures taken at a standard magnification using a binocular microscope with an attached camera. Due to a change in the microscope-software, that occurred after measuring head width but before calibrating pixels to the mm scale, we cannot be certain that the obtained conversion factor fit the settings used before the software update. Our calculated head widths therefore seem small in comparison to those measured in other studies (Boomsma et al. 1982). Regardless, the differences between Exp1 and Exp2 cohorts are unaffected, and robust.

Statistical analysis

Ages at death in days were calculated using the date the callows were collected from their natal colonies as birthdate and their reported age at death. Individuals that died due to accidents or that were alive at the end of the study were treated as right-censored. Cox proportional hazards regression analysis was used to analyze differences among cohorts and between treatments (Exp1, Exp2).

It has been shown for honey bees (Rueppell et al. 2009) that colony size affects worker survival. To investigate the effect of experienced cohort size on mortality, we investigated the effect of experienced cohort size on mortality. We calculated the probability that an individual would die within a time interval, given that the individual was alive at the start of the interval. In survival analysis this probability is called “hazard” (Klein and Moeschberger 2003). We set the interval to 100 days and calculated the hazards dependent on age and cohort size at the start of the interval using the Kaplan-Meier product-moment estimate as implemented in the R package *survival* (Therneau and Lumley 2011). We then examined the effects of cohort size, age and the origin of workers on the hazard-rate. In effect, we performed a set of general linear models on the log-transform of the hazard rate, thereby assuming Gompertz mortality (Klein and Moeschberger 2003). We used the AIC criterion to find the best model.

All statistical analysis were performed using R-statistical software (R Core Development Team 2010) and the packages *survival* (Therneau and Lumley 2011), *MaxLik* (Toomet and Henningsen 2010).

Results

Head width

Workers from small colonies (Exp1) had a mean head width of 0.69±0.05 mm (N=57). Workers raised in larger colonies (Exp2) (head width: 0.74±0.04 mm, N=50) were significantly larger than the workers from Exp1 (t-test: p<0.001, df=105, t=-5.35) (fig.1).

Survival

Workers from different sized colonies showed significantly different survival rates. The Cox proportional hazard regression model showed that workers from larger, later colonies (Exp2) lived significantly shorter than workers produced by small, early colonies (Exp1) (n=390, events=293, coefficient=-0.678, p<0.001), while the initial sizes of the experimental cohorts showed no effect. The exponentiated coefficient is interpretable as multiplicative effect of the experiment on the hazard and showed a 49% increase in survival for individuals in Exp1 at each time period (day). Plotted Kaplan-Meier product-moment estimates are shown in fig. 2. Clearly, workers derived from small colonies seem to experience a lag of mortality for about 200 days and appear to survive for longer time. This pattern is confirmed in the hazard analysis: log hazard during a 100 day interval could be sufficiently explained by a model including cohort size only (cohort size: coefficient = - 0,0279, $p<0,05$), indicating that the probability of death within the next 100 days increase with decreasing cohort size. However, when the effect of the age of workers on the log hazard was analyzed, it mattered whether workers were raised in early, small or late, large colonies (age: coefficient = 0,00186, $p<0,05$; Exp2 coefficient 0,254, $p<0,05$). In conclusion, workers from early, small colonies had a higher probability of attaining older ages. However, when artificial experimental cohorts started to shrink, diminishing cohort sizes affected workers from early, small and late, large colonies in a similar way.

We additionally calculated mean and maximum worker life span. The reported values represent a minimum estimate of the mean and a minimum estimate of maximum life span since 6.5% (Exp1) and 17.9% (Exp2) of the workers were still alive at the end of the study. (Exp 1: mean life span > 535.98±233.71 days, maximum life span > 951 days; Exp 2: mean life span > 290.32±162.38 days, maximum life span > 622 days).

Discussion

In this study we showed that both size of workers and worker quality, measured as life span of artificial cohorts in laboratory conditions, are determined by the state of the colony the workers are reared in: young and small colonies produced smaller and longer-lived workers than more mature and larger colonies. In addition, we could show that life span was affected by actually experienced cohort size (social environment). This effect did not differ among experiments and was less pronounced than the differences between the experiments.

Mailleux et al (2003) showed before, that small *Lasius niger* colonies produce smaller workers than larger colonies. In a similar social environment the workers derived from small colonies showed a longer life span than the workers that hatched in larger colonies. Considering further, that experienced cohort size had no differential effect on workers reared at different stages throughout colony development, which deploy different foraging behavior, we conclude that life span of workers was determined by effects that originate from the natal colony, rather than the experienced social environment.

Nevertheless, we found a negative correlation between worker size and life span has previously been reported for *O. smaragdina* and *A. subterraneus* workers (Camargo et al. 2007; Chapuisat and Keller 2002)(fig. 2).

Our results in comparison with other studies on worker life span

Information on worker life span in ants is generally scarce, and this is the first study that compared the life span of a monomorphic ant worker caste from different developmental stages of the colony. In several studies mean or maximum life span of worker for a given species can be found (Fowler et al. 1986; Tsuji et al. 1996; DuBois 2000; Hölldobler and Wilson 1990; Haskins and Haskins 1980; Hartmann and Heinze 2003). Just a few studies followed individual workers to gain information on the survival of individuals or to compare worker survival of different castes (Schmid-Hempel and Schmid-Hempel 1984; Chapuisat and Keller 2002; Calabi and Porter 1989). The only previous information on *L. niger* worker life span was that it ranges between one and two years (Hölldobler and Wilson 1990). Our results showed a maximum life span of more than 2.6 years under laboratory conditions and a mean life span of 536 days (Exp1) or 290 days (Exp2) (note that some individuals were still alive at the end of the study). This worker life span is comparable to different species of the genera *Myrmecia* and *Myrmica* (Haskins and Haskins 1980; Hölldobler and Wilson 1990) but is longer than that of workers in the genus *Monomorium*, which display a maximum life span of several weeks up to 1.4 years (DuBois 2000; Peacock and Baxter 1950). Species of the genera *Formica* and *Temnothorax* on the other hand have a reported maximum life span of several years (Bierwirth 2003; Hölldobler and Wilson 1990).

Body size and life span

Effects of body size on life span within social insects are mixed. For the red fire ant (*Solenopsis invicta*) a positive association of body size and life span was shown and has been linked to the different metabolic rates (larger worker have a lower metabolic rate per unit of mass), which would also explain temperature effects on worker life span (Calabi and Porter 1989). In this study temperature was held constant but metabolic rates were not measured. Our study showed a negative association of life span and body size. This finding might indicate a trade-off between body size and life span of workers as found in within species comparisons outside the social insects in dogs and mice (Patronek et al. 1997; Miller et al. 2002). The size and growth rate during larval development is under control by the natal colony and presumably has a strong effect on the demographic properties of the individuals. The found negative correlation between worker size and life span has previously been reported for *O. smaragdina* and *A. subterraneus* workers, which both showed lager morphological differences than our cohorts but less differences in survival probability or mean life span as compared to the differences between our experiments (Camargo et al. 2007; Chapuisat and Keller 2002) (Fig 2). In both studies small workers performed different tasks than large workers and consequently experience different levels of extrinsic mortality (Chapuisat and Keller 2002). Thus, the extended life span of workers produced in small colonies with individual foraging may have evolved as a response to reduced extrinsic risk, rather than a response to body size and reduced costs of growth. In fact, smaller workers of *L. niger* produced early in colony development have been shown to be more efficient for brood production than larger workers that are more efficient at foraging. This may point to a task specialization of the different worker cohorts which may be accompanied by differences in extrinsic mortality (Porter and Tschinkel 1986; Mailleux et al. 2003). As we were unable to find any evidence for changes in extrinsic risk with colony stage or size in the literature, we cannot be sure whether the differences in worker life span in the course of colony growth have evolved as a response to extrinsic mortality risk. More studies on the interaction between colony size, foraging mode and extrinsic mortality would be desirable to establish the link between extrinsic mortality and the evolution of worker life span in the social insects.

Allocation shifts during early colony growth

Apart from a proximate trade-off between body size and life span, the results of our study may also be caused by ultimate factors, such as a high demand of the colony for early produced workers. During independent and claustral colony foundation, the colony faces a trade-off between worker number and worker size, with the size of the young colony having strong effect on the colony’s survival probability (Oster and Wilson 1978). The energetic costs of different size workers can be separated into costs associated with worker maintenance (metabolism) and production (Calabi and Porter 1989). Therefore, it seems to be essential for the colony to increase investment into maintainance of workers in early stages of the colony. Hence, the colony should produce a higher number of smaller and longer lived workers to improve the survival probability and growth of the colony as long as the colony is below the threshold size where more complex cooperative foraging is feasible (Mailleux et al. 2003). Additionally, changes in worker behavior from risk-sensitive (at stages where individual workers are more important) to risk-prone worker strategies may appear at the transition to cooperative foraging, because the loss of a single individual is a less severe hazard for larger colonies (Strassmann 1985; Rueppell et al. 2009).

Why should a colony produce bigger workers if these live shorter? Diverging aging trajectories of different castes could be an outcome of ergonomic optimization which could also be important in species with monomorphic worker castes (Tschinkel 1993; Oster and Wilson 1978; Schmid-Hempel 1992). The production of larger workers increases the amount of resources allocated into the production of a single individual (Calabi and Porter 1989). This in turn leads to higher losses per individual in case of a death caused by predation if compared to the loss of a smaller worker. The production costs of each larger worker could be counterbalanced by a reduction of maintenance costs for larger workers which, as in *Solenopsis invicta,* may have a lower metabolic rate (Calabi and Porter 1989). Additionally, larger *L. niger* workers are more efficient foragers and thus the amount of resources obtained at each foraging trip increases (Mailleux et al. 2003). These changes could ultimately change the cost benefits ratio of each worker.

As discussed above, it has been shown that both worker size and colony size may have effects on worker life span (Calabi and Porter 1989; Rueppell et al. 2009). Unfortunately, we could not disentangle the effects of age and size of the natal colony. Therefore, we cannot predict the demographic properties of workers raised in older colonies that have been reduced in size.

In conclusion, our results may indicate a trade-off between body size and life span of workers leading to longer lived but smaller workers. Alternatively, with the switch to cooperative foraging (Mailleux et al. 2003), which is accompanied by the production of larger workers, the quality of the worker might change caused by a task specialization and the different levels of extrinsic risk faced by the workers that originated from different colony stages.

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Figures

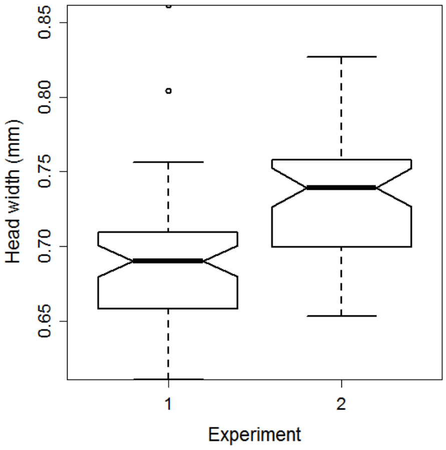

Fig. 1 Notched box plot showing the head width of the different experimental groups. Head width between experiment 1 and experiment 3 was different (t-test: p<0.001, df=105, t=-5.35).

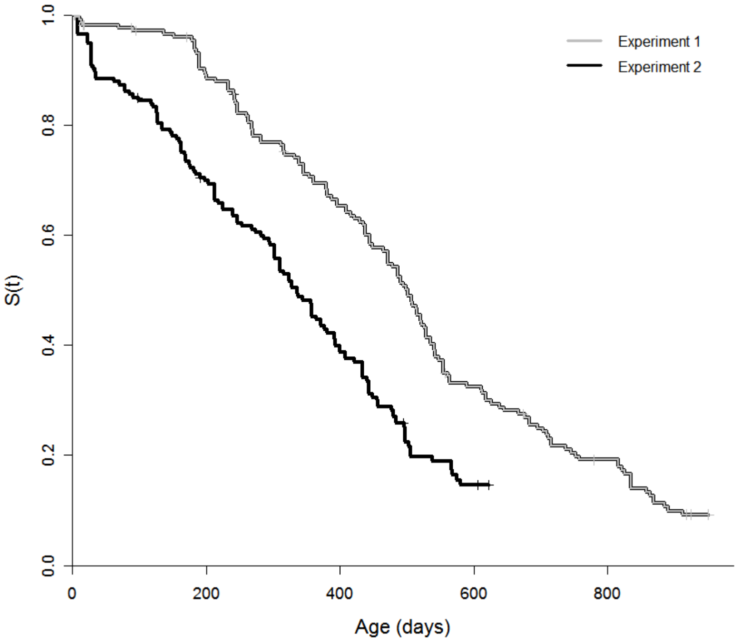

Fig. 2 Survival of workers born in different sized colonies. The grey line represents workers from small colonies (Exp1) and the black line represents workers from larger colonies (Exp2) which lived significantly shorter (Cox-ph: n=390, events=293, coefficient=-0.678, p<0.001).

Chapter III: Colony size explains the life span differences between queens and workers in eusocial Hymenoptera

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Abstract

Eusocial Hymenoptera show a unique divergence in life span of queens and workers, queens belong to the longest lived insects while workers in most eusocial species have significantly shorter lives. The different phenotypes within a colony emerge through reproductive division of labor, which is a characteristic trait of eusocial animals. Division of labor as a measure of organismal complexity increases with colony size in eusocial species similar to the increase of complexity with size that has been shown for the whole range of living organisms. We show that queen and worker life span diverge in closely related species representing the transition from solitary to social life and show that queen and worker life span are correlated if colony size is taken into account: with increasing colony size the life span differential between queen and worker increases, whereas neither queen nor worker life span is associated with colony size. Additionally the life span differential is better explained by colony size than by the weight differences between the castes. The divergence of phenotypes found is in line with the increasing specialization of subunits in larger organisms, which leads to increasing complexity. We argue that division of labor is acting to increase colony efficiency, which in turn shapes the investments made into individuals leading to short lived workers and long lived queens. Additionally maintenance investments may be shaped due to the variable extrinsic risk faced by different castes.

Keywords: *colony size - division of labor* **-** *life span – queen - social insect – worker – eusocial - hymenoptera*

Introduction

Biology is largely a matter of size: larger organisms tend to be more complex and this rule applies to the whole range of living organisms and to different levels of biological organization (Bonner 1988, 2004; Bell and Mooers 1997; Changizi et al. 2002). Often organismal complexity is measured as the number of specialized subunits (Bell and Mooers 1997) but it may also be measured by their phenotypic divergence (Changizi et al. 2002). The increase in complexity results from increasing cooperative division of labor that appears at all levels of biological organization and which is a measure of the degree to which subunits (organelles, cells and individuals) specialize on different tasks and thus reflects complexity (Gadau et al. 2009; Bonner 2004; Rueffler et al. 2011). Division of labor benefits the organism with increasing efficiency because it allows the organism to simultaneously accomplish several physiological processes (Simpson 2012; Hurd et al. 2007). Once formerly autonomous units in a group shift to be intrinsically dependent upon one another they become integrated into a new hierarchical layer of biological organization representing an evolutionary transition such as that from unicellular to multicellular life or that from solitary to social life, which are accompanied by a shift in the level of selection (Smith and Szathmary 1997; Goldsby et al. 2012; Heylighen 2000; Wilson and Wilson 2007).

This latest evolutionary transition is accomplished by eusocial species (Michod 2000). The emerging colonies have therefore been termed superorganisms, a collective organism consisting of many individual “sub-unit” organisms (Wilson and Sober 1989; Wheeler 1911) but see Strassmann & Queller (2009). The level of the superorganism is reached when societies are bound by altruism and division of labor (Gadau et al. 2009). The colony can thus be likened to the body of an individual organism. As a result of division of labor, the colony is composed of reproductive individuals (queens) that represent the germ line and workers that represent the soma, but as compared to a multicellular organism with lower genetic relatedness between its members, which gives raise to conflict and competing levels of selection (individual- and colony-level) (Boomsma 2009; Ross and Matthews 1991; Wilson and Sober 1989; Wheeler 1911).

Here we focus on queen-worker differences in life span that arise with changes in colony size. Increasing colony size has a number of consequences for the social organization and the evolution of life histories of different individuals within eusocial colonies, which are driven by increasing division of labor (Bourke 1999; Dornhaus et al. 2012). The positive association of colony size and division of labor leads to increasing morphological skew as the degree of task and caste specialization increases, as well as a divergence of life span between queens and workers and the loss of individual totipotency (e.g. reduced reproductive capabilities of workers), all of which increase efficiency gains for larger colonies (Bourke 1999; Hurd et al. 2007; Alexander et al. 1991; Carey 2001; Schmid-Hempel 1998; Hölldobler and Wilson 1990; Changizi et al. 2002). Once group living has evolved, coevolutionary changes affecting life span evolution emerge (Alexander et al. 1991; Carey 2001). Disruptive selection become apparent once the chance or physiological ability of worker reproduction is minimized and leads to the evolution of distinct worker and queen phenotypes (Bourke 2007). The divergence of phenotypes is a self-reinforcing process because early aging in helpers with increasing colony sizes decrease the chance of future reproduction, promoting further phenotypic specialization (Alexander et al. 1991). This leads from a reduced reproductive potential to complete worker sterility while the fertility and life span of the queen increases (Hölldobler and Wilson 1990). As a result, queens belong to the longest lived insects and generally live longer than workers, although in most species they do not differ genetically and develop from the same female larvae (Heinze and Schrempf 2008; Keller and Genoud 1997) but see Kerr (1950).

However, beside the effect of colony size on life span evolution, life span in the social insects may also be correlated with body size (Porter and Tschinkel 1985; Calabi and Porter 1989), independent of size as a response to the level of extrinsic mortality (Chapuisat and Keller 2002) or may be task dependent (Rueppell et al. 2007). Therefore both evolutionary (ultimate) and mechanistic (proximate) theories of aging could explain the divergence of queen and worker life span (Hughes and Reynolds 2005). Evolutionary theories of aging assume that high levels of extrinsic mortality select for a reduced investment in physiological maintenance leading to a shorter life span (Medawar 1952; Williams 1957; Hughes and Reynolds 2005). Following this the evolution of the observed life span variation between different castes in social insects has been linked to the caste specific differences of extrinsic mortality (Parker 2011; Heinze and Schrempf 2008; Keller and Genoud 1997). Evolutionary selected worker life span as a response to different levels of extrinsic mortality has been shown to be preserved under laboratory conditions (Chapuisat and Keller 2002). Extended longevity, by contrast, is associated with adaptations that reduce the risk of predation. Examples of that are subterranean living (Buffenstein 2005), the ability to fly (Austad and Fischer 1991), group or social living (Carey and Judge 2001; Keller and Jemielity 2006; Buffenstein 2005; Carey 2001) and intergenerational transfers (Amdam and Page 2005; Lee 2003). In most cases the queens in eusocial colonies are long-lived, because they are protected in the center of the colony while short-lived workers take over tasks that are correlated with higher levels of extrinsic risk (Schmid-Hempel 1998). As a result the mean life span of queens in *Solenopsis invicta* is 30 times longer than that of small workers (Calabi and Porter 1989; Hölldobler and Wilson 1990). Even species with less pronounced morphological caste differences such as *Diacamma rugosum* show a 1.3 fold difference of maximum life span between workers and queens (Tsuji et al. 1996). In contrast, mechanistic theories of aging make predictions about the relationship of metabolic rate and life span. Larger organisms have a lower metabolic rates per unit of mass, a slower development and greater life span (Finch 1990; Collatz and Sohal 1986). For example, the life span of fire ant workers (*Solenopsis invicta*) is affected by both temperature and body size, which both change the metabolic expenditure (Calabi and Porter 1989).

However, mechanistic and evolutionary theories of aging have been broadly discussed (Parker 2011; Jemielity et al. 2005; Keller and Genoud 1997; Heinze and Schrempf 2008; Moret and Schmid-Hempel 2000; Schmid-Hempel and Wolf 1988) and therefore in this study we elaborate the effect of colony size on changes in life span for both workers and queens. Highly eusocial species with larger colonies tend to have shorter lived workers (Schmid-Hempel 1998; Matsuura 1991) but for queens no such association was found (Hölldobler and Wilson 1990).

We first review trends in life span evolution associated with the transition from solitary to eusocial organization displayed by closely related species that show both solitary and social organization. Because within the Hymenoptera only bees and wasps represent this transition, we also included other eusocial species (mole-rats, gall inducing thrips and ambrosia beetles) in this analysis. This approach can give an indication on how life span changes with the onset of task specialization of different individuals within a group.

Second, because colony size is correlated with social complexity and division of labor, including reproductive potential and caste differentiation, we tested the hypotheses that colony size can explain the differences between queen and worker life span within the eusocial Hymenoptera as proposed by Carey (2001). As phenotypic specialization increases, the life span differential between queens and workers should increase as a result of disruptive selection. Disruptive selection should lead to the evolution of diverging caste specific life span as a response to task specific levels of extrinsic mortality. Additionally, because the differential life span of workers and queens emerges from group living and the associated reproductive division of labor we expect that the differences between worker and queen life span should be phylogenetically independent and apply to terrestrial (wasps, bees) as well as subterranean (ant) species similarly as a response to colony size. Alternatively life span differences between worker and queen castes could result from the increasing morphological differences caused by increasing division of labor in larger colonies which in turn affect metabolic rate and longevity. If so, we should find a correlation between the differences in life span and the differences in weight between queen and worker castes.

To analyze the association between colony size and life span we collected life span, colony size and dry weight data on eusocial Hymenoptera from the published literature.

Methods

Data collection

Generally data on survival in social species were scarce. Apart from a few comparative studies or book chapters that focus on life span or colony size (Keller 1998; Beckers et al. 1989; Hou et al. 2010; Hölldobler and Wilson 1990; Schmid-Hempel 1998; Page Jr and Peng 2001), data needed for this study were often hidden in papers focusing on other topics. We collected data for eusocial Hymenopterans on life span, colony size and dry weight by searching the scientific literature. We conducted queries on Google scholar and ISI web of knowledge for the following keywords “life span”, ”survival”, ”colony size” and “worker/queen weight”. The collected data originated from a combination of laboratory, field observation and anecdote (especially the data on life span). Sources for the data used in our analysis are given in the supplemental material (table S2).

Life span

We collected life span data of solitary and closely related species that represent the steps from solitary towards eusocial organization as well as life span data for eusocial Hymenoptera. Within the Hymenoptera only bees and wasps represent recent examples of the transition from solitary to eusocial organization and thus we added eusocial species apart from the Hymenoptera (mole-rats, thrips and ambrosia beetles) to analyze life span changes with changes in social organization. While data on survival would be more appropriate but rare we had to use data on mean or maximum life span, which was often complicated because sample sizes especially for queens were often low. Another problem was that published data can be obtained from laboratory or field colonies. For queens the difference between laboratory studies and field studies should be minimal; in both cases the queen lives in the center of the colony protected from extrinsic risks. In the case of the workers laboratory life span could be higher than for field studies but bees and wasps were often allowed to forage outside in laboratory studies. Because we were interested in the intrinsic life span differences shaped by natural selection, which in turn result from differential investments into individuals or castes as a result of different levels of extrinsic mortality (Chapuisat and Keller 2002; Heinze and Schrempf 2008), we preferred laboratory over wild data, if both were available (e.g. *Harpagoxenus saltator*). Wild data especially for workers were often left truncated and did not cover the whole individual life span (e.g. workers of *Pogonomyrmex barbatus* were just monitored after first appearance outside the colony). We omitted such data from the analysis. In two cases field data were used because both queen and worker life span were available as field data but for just one caste as laboratory data and otherwise we would have mixed wild and laboratory data for one species, which we avoided if possible (e.g. *Vespa simillima*). If several maximum life span values were obtained we used the highest reported value because maximum life span usually represents maximum observed life span, which may vary between studies mainly depending on sample size. For species with polymorphic worker castes with differing life span (mean or maximum life span) we used the highest reported values because they would represent maximum worker life span (e.g. *Acromyrmex subterraneus brunneus, Solenopsis invicta, Apis mellifera* diutinus workers); if information was only available for one caste we used this. If survival curves were published we calculated mean life span and used the age of the last survivor as maximum life span. When just life expectancy was reported (e.g. *Bombus ferdivus*) but no survival curve was published we used the reported value as mean life span. If the reported maximum life span value was obtained while the individual was still alive (LS>3 years) we used the reported value (LS=3 years) to avoid an overestimation of life span. Sometimes we just found estimates of queen life span derived from colony survival, which may be critical as unobserved queen turnover may have occurred (Keller 1998). For some annual species life span was reported after the first egg was laid or colony life span alone is reported, so we then added the hibernation time, which is part of the adult life span (e.g. *Bombus melanopygus*). If a life span range was given we used the upper limit as maximum life span and the midpoint as mean life span (e.g. *Tetragonisca angustula*).

We collected mean queen life span of 72 species. Mean worker life span was collected for 82 species. Maximum life span was obtained for 79 species (queen) and 76 species (worker).

Colony size

Colony sizes were collected from the published literature (table S2 in the supplemental material). If possible we used mean colony size of mature colonies. In cases where we just found one value we used that. If we found several reported colony sizes we took the mean, and if several mean values were obtained we used the mean weighted by the sample size (e.g. *Pogonomyrmex occidentalis*). If a range of colony sizes was given we used the midpoint of the reported values, and in cases where just a minimum size was given we used this. If we found a statement that the colonies are smaller than a certain value we reduced this by one third because we found that average values are 20-50% smaller than reported maximum values, and additionally we did not want to overestimate colony size.

Dry weight

To account for the morphological differences between queens and workers we collected dry weight data for each caste. If we obtained several dry weight measures from different studies or several values for different worker castes we calculated the mean dry weight across castes and studies. Data sources can be found in table S2 in the supplemental material.

Data analysis

Mean and maximum life span calculation

For some species we collected both mean and maximum life span data but for others we obtained just mean life span for worker and maximum life span of queens or vice versa. To compare those species and to incorporate a higher variability of species into our analysis we calculated a conversion factor for both queens and workers to calculate the mean from maximum life span or vice versa. Instead of dividing maximum life span by 1.45 to obtain mean life span (Keller and Genoud 1997; Hou et al. 2010) we used a conversion factor retrieved from a linear regression (weighted by the study sample sizes) between mean and maximum life span of species where we collected both life span measures for either queens or workers. We chose linear regressions through the origin because of the clear relation between mean and maximum queen life span (as seen in fig. 1). For the regression we only used data from species where both mean and maximum life span were obtained either from the laboratory or the wild. For queens the conversion factor (slope) obtained was 1.34±0.05 ($t_{1,44}$

= 28.32, $P < 0.001$, $R^2 = 0.95$, $F = 802.2$) and for workers 1.66±0.07 ($t_{1,55} = 21.15$, $P < 0.001$, $R^2 = 0.89$, $F = 447.3$) (Fig. 1). After calculating maximum life span from mean life span (Fig. 1) we used the maximum life span data for all correlations between life span and colony size; using mean life span revealed similar results.

Life span and colony size

We used linear models to analyze the relationship between worker as well as queen life span and colony size. To test for a correlation between queen and worker life span we calculated the ratio of queen and worker life span (queen life span in days / worker life span in days) to obtain a dimensionless measure. We used linear regressions to analyze the relationship between the life span ratio and colony size. To normalize the residuals of the linear models we had to log-transform both the life span ratio and colony size. The species used and data sources can be found in table S2 in the supplemental material.

For the Formicidae we also compared models including the mode of colony founding (dependent/independent) or queen number (monogyny/polygyny) along with colony size as explanatory variables. We used a backward elimination modeling approach starting with a maximal model. We then eliminated stepwise non-significant terms until a minimum adequate model with significant terms was reached (Crawley 2002). We used ordinary least squares (OLS) linear models and model comparisons were performed using ANOVAs with χ^2 tests. Additionally, the models were compared using the Akaike information criterion (AIC).

Dry weight ratio

We were able to calculate the weight ratio (queen weight / worker weight) for 15 species, for which we also had the data to calculate the life span ratio (queen life span / worker life span).

We were then able to test if either colony size or the dry weight differences between queens and workers can explain the life span differences between queens and workers.

We used a backward elimination modeling approach starting with a maximal model with life span ratio as the response variable and dry weight ratio and colony size, plus the interaction between them, as explanatory variables. We then eliminated stepwise non-significant terms until a minimum adequate model with significant terms was reached (Crawley 2002). We used ordinary least squares (OLS) linear models forced through the origin with log-transformed variables. Following a Lilliefors- (Kolmogorov-Smirnov) test the residuals were found to be normally distributed for all models. Model comparisons were done using ANOVAs with χ^2 tests. Additionally the models were compared using the Akaike information criterion (AIC). The used species and data sources can be found in table S2 in the supplemental material.

Comparative analysis

We used phylogenetic independent contrast (Felsenstein 1985; Garland et al. 1992) for continuous variables to control for evolutionary relationships. Contrasts for continuous variables were obtained using the crunch option within the R- package caper (David et al. 2012) and were then subjected to regression analyses through the origin.

We used two different approaches. First we used an existing phylogeny for the Fomicidae (Moreau et al. 2006), which includes some species that appear in our dataset. We further added species if the genus was included in the existing phylogeny. We were able to include 13 ant species, the honey bee and a wasp species as these where used as outgroups in the analysis of Moreau et al. (2006).

Second, we constructed a phylogeny from published trees of social Hymenoptera (Arevalo et al. 2004; Moreau et al. 2006; Costa et al. 2003; Schmitz and Moritz 1998;

Danforth 1999). Because no information on branch lengths could be assigned to our composite phylogeny we set all branch lengths to the same value (Fjerdingstad and Crozier 2006). Branch length information is not indispensable for independent contrast analyses, which are generally robust (Garland et al. 1992). We were able to include 29 species representing all genera in our analysis (the used tree and species can be found in the supplemental material (table S1, figure S1)).

Statistical analysis

All statistical analyses were performed using the R-statistical software (R Core Development Team 2010) and the packages nortest (Gross and Ligges 2012), caper (David et al. 2012) and ape (Paradis et al. 2004).

Results

Life span changes during social evolution

In our review of the literature an increased divergence of life span of queens versus workers with increasing sociality was found in all groups examined (including mole-rats, thrips and ambrosia beetles in addition to bees and wasps, which represent the social Hymenoptera).

For African mole-rats (*Bathyergidae*) maximum recorded life span of solitary mole-rats was between 6 (*Bathyergus suillus*) and 11 (*Georychus capensis*) years (Dammann 2006), but data were scarce and hence not as reliable as data for the social species. Maximum life span in the social species varied from 11 years (*Cryptomys hottentotus*) to 30 years (*Heterocephalus glaber*) (Dammann 2006; Liang et al. 2010). Non-breeders of the genus *Fukomys* displayed significant differences in life span compared with breeders. Non-breeder life span was comparable to solitary species (*Fukomys anselli,* maximum life span= 8 years;

Fukomys mechowii, maximum life span= 10 years) while breeder life span was prolonged (*Fukomys anselli:* maximum life span= 19 years, *Fukomys mechowii*: maximum life span= 16 years) (Dammann et al. 2011; Dammann and Burda 2006). Breeders and non-breeders of *Heterocephalus glaber*, which forms the largest groups (up to 295 individuals) within the social mole-rats, showed similar life span (Buffenstein 2008; Liang et al. 2010; Buffenstein 2005; Brett 1991).

Several gall-inducing thrips are eusocial (Kranz 2005; Crespi 1992) and in the genus *Kladothrips* both solitary and eusocial species exist (Choe and Crespi 1997). In solitary species (*Kladothrips rugosus* and *K. ellobus*) females lived about 10-12 months (annual life cycle), and the same life span was found for foundresses in the eusocial species *K. intermedius* and *K. waterhousei,* while soldiers in the same species showed a reduced life span of about 3-6 months (Crespi et al. 2004).

In the solitary fungus-growing ambrosia beetles *Dolipygus dubius* and *Dendroplatypus impar* offspring leave the nest after emergence and individuals had life spans of 14-16 weeks (Browne 1962) and 1 year (Browne 1961). Species in which cooperative breeding occurs, such as *Notoplatypus elongates,* reached a life span of more than 3 years (Kirkendall et al. 1997). The only known eusocial species is *Austroplatypus incompertus*, which forms monogynic colonies that can last up to 37 years (Costa 2006). Females in this species may live more than 4 years while it was not known how long the workers live (Kirkendall et al. 1997).

In the superfamily Apoidea eusociality has arisen at least eight times (Wilson 1971). Solitary Apidae (*Ceratina calcarata*) had an average life span of approximately 14-16 months and a maximum life span of almost 2 years (22 months) (Rehan and Richards 2010b, 2010a). In facultatively social Apidae (*Xylocopa virginica*) mean life span was below 11 months and maximum life span reached 26 months (Richards 2011).

Wasp life span increased with increasing social complexity (Carey 2003; Wilson 1975; Evans 1958). Solitary parasitoids had short life spans of 14-60 days (*Natocyphus*), extensive parental care in *Gorytes* and *Stenogaster* species led to an increase in life span (60-365 days) leading to advanced eusocial wasps (e.g. *Vespula*) with queen life spans ranging from 180-1000 days (Evans 1958; Wilson 1975; Carey 2003). However, worker life span in eusocial wasps (*Vespula, Vespa*) seemed to be similar to their related solitary species (20-65 days) (Strassmann 1985). Data on eusocial wasps was also included in the following analyses.

Worker life span and colony size

We did not find a general trend in the relationship between maximum worker life span and colony size. All regressions of maximum worker life span on colony size showed significant intercepts but not slopes (table 1; fig. 2). Due to the small sample size the Halictidae were only included in the regression on all Hymenopterans.

Queen life span and colony size

We did not find a general trend in the relationship between maximum queen life span and colony size. The regressions of maximum queen life span on colony size showed significant intercepts except for the Apidae; slopes were marginally significant for the Formicidae, Apidae and all Hymenoptera combined (table 1). In the Apidae the $R^2 = 0.761$ was large given the small sample size of five species. Long-lived queens were found at all colony sizes while shortlived queens do not appear at larger colony sizes (fig. 3). Due to the small sample size the Halictidae were only included in the regression on all Hymenopterans.

Life span ratio (queen / worker) and colony size

We found a positive association between the life span ratio, which represents the differences in the life-history trait life span between queens and workers and colony size, as follows:. Hymenoptera: estimated slope= 0.23±0.01 ($t_{1,48}$= 17.14, P<0.001, R^2=0.86, F=293.7). Apidae: estimated slope= 0.33±0.3 ($t_{1,4}$= 6.92, P=0.002, R^2=0.92, F=47.92). Vespidae: estimated slope= 0.32±0.04 ($t_{1,9}$= 7.74, P<0.001, R^2=0.86, F=59.87). Formicidae: estimated slope= 0.21±0.01 ($t_{1,30}$= 16.55, P<0.001, R^2=0.9, F=273.7). Figure 4 shows the results for all Hymenoptera combined. For the Formicidae we also compared models including the mode of colony founding (dependent/independent) or queen number (monogyny/polygyny) along with colony size as explanatory variables. Neither of the additional variables was significant and the model just including colony size was preferable (ANOVA (queen number): change in the residual sum of squares= -0.530, d.f. = 1 on 46, p= 0.22; ANOVA (mode of colony founding): change in the residual sum of squares= -0.114, d.f. = 1 on 24, p= 0.867). The AIC was lower for the model using colony size as main effect (AIC = 4.81) than the model with colony size and queen number as explanatory variables (AIC = 12.78) or the model including colony size and the mode of colony founding (AIC = 13.02).

Independent contrast

Using the phylogeny of Moreau et al. (2006) revealed a slope of 0.2 ± 0.063 ($t_{1,13}$ = 3.17, P = 0.007, R^2 = 0.44, F = 10.04). Using the tree created by merging different Hymenoptera revealed: slope = 0.21 ± 0.047 ($t_{1,24}$ = 4.38, P < 0.001, R^2 = 0.44, F = 19.18). In both cases the relationship between the life span ratio and colony size did not change when controlling for phylogenetic effects.

Life span ratio and weight ratio

After elimination of non-significant terms, only colony size remained in the model (slope = 0.21±0.02, $t_{1,14}$ = 11.07, $p<0.001$, $R^2 = 0.89$, F = 122.6). Both the interaction between colony size and dry weight ratio (ANOVA: change residual sum of squares = 0.086, d.f. = 1 on 13, p = 0.2961) and dry weight ratio as a main effect (ANOVA: change in residual sum of squares = 0.004, d.f. = 1 on 14, p = 0.8552) were not significant.

We also compared the Akaike information criterion (AIC) values of the minimum adequate model (with colony size as the only explanatory variable) with that of a model with dry weight ratio as the only explanatory variable (slope = 0.57±0.09, $t_{1,14}$ = 6.42, $P<0.001$, $R^2 = 0.73$, F = 41.16). The AIC was much lower for the model using colony size as the explanatory variable (AIC = 6.36) than the model with only dry weight ratio (AIC= 19.97). This is a strong indication that the model with colony size is the better model to explain the life span ratio of queens and workers than the model including the weight differences of queens and workers.

Colony size and weight ratio

We found a positive association between colony size and weight ratio using colony size as the explanatory variable: estimated slope = 0.31±0.04 ($t_{1,14}$ = 8.69, $P<0.001$, $R^2 = 0.83$, F = 75.62).

Discussion

Our comparative study reveals a correlation between division of labor measured as colony size and the life span ratio of queens and workers in eusocial Hymenoptera (fig. 4). The differences in life span increase with increasing colony size and point to a correlation between queen and worker life span when colony size is taken into account. Interestingly the correlation with colony size is weak or disappears if workers and queens are considered independently (table 1; figs. 2,3). While weight differences between queens and workers increase with colony size, colony size itself is a better predictor for the life span differences found.

Throughout the transition from solitary to eusocial organization across different species from insects to vertebrates we find a divergence in life span between castes with increasing sociality, as did Carey (2001), probably caused by increasing reproductive division of labor (Bourke 1999). In most groups life span of the reproductive individuals increases while worker life span stays within the range of the comparable solitary species. However, we found two exceptions to this pattern. First, soldiers of the gall-inducing thrips show a reduced life span as compared with solitary species while the foundress life span remains constant. Another unique pattern is that soldiers in this species are larger than foundresses, while their main task is to defend the colony (Perry et al. 2004). Second, within the social mole-rats, *Heterocephalus glaber* is an exception by showing the same life span of breeders and non-breeders. This may be caused by differences in maintaining the social system between the *Fukomys* species (incest avoidance) (Burda 1995) and *Heterocephalus glaber* (dominant control), where the queen suppresses other colony members from reproduction but workers retain the ability to reproduce within their natal colony (Faulkes and Abbott 1996).

However, when focusing on the social hymenoptera we find no correlation of worker life span with colony size (fig. 2). Worker life span might decrease with colony size as in wasps but only within closely related species (Matsuura 1984). Thus, phylogenetic constraints could drive the results of Mastuura (1984) and have also been suggested for the long life span of *Myrmecia* and ponerine workers (Hölldobler and Wilson 1990). Carey (2001) argued that worker life span or daily birth rate determine colony size, either of which needs to increase in order to obtain larger colonies (Carey 2001). Because worker life span does not change with colony size the egg-laying rate of the queen must increase with increasing colony size.

We found marginally significant correlations of queen life span with colony size for the Formicidae and the Apidae (fig. 3, table 1). Within the Apidae positive association between colony size and queen life span might exist but with our small sample size we cannot statistically confirm this. Within the Formicidae long queen life span occurs at all colony sizes while short-lived queens are only found in smaller colony sizes (fig.3). As pointed out by Keller & Genoud (1997) queens in polygyne colonies live significantly shorter than queens of monogyne species and for our dataset queen number would be a better predictor of queen life span than colony size. Interestingly, polygyne species appear with higher frequencies at smaller colony sizes. Short-lived queens do not occur in species with large colony sizes; this can either result from ancestral monogyny (Schrempf and Heinze 2007) with the need for a longer life span in queens with larger colonies or it could be caused by a sampling bias because queen life span can be obtained by colony survival in monogyne but not in polygyne species (Gordon 1991; Hou et al. 2010). Larger colony sizes lead to an increased protection of the queen against extrinsic risks. Additionally, an increasing reproductive value and the dependency of colony fitness on the survival of the single queen should select for an increasing queen life span and survival, which is in line with the evolutionary theories of aging (Keller and Genoud 1997; Stearns 1992).

If we could control for ecological factors or larger phylogenetic trends within the hymenoptera it might be possible that a correlation between life span (queen and worker) and colony size would appear. For example queen life span of subterranean species (ants) is much greater than for terrestrial species (bees and wasps) (Carey 2001) and colony size is likely to be set by ecological factors (Bourke 1999). The relative differences of life span (life span ratio) and colony size used here are likely to control for phylogenetic and ecological constraints and offer an opportunity to compare different eusocial species as the result of the independent contrast analysis suggests. In our comparison bee, wasp and ant species fit into the observed relationship of life span ratio and colony size. We could even include the social mole-rats of the genus *Fukomys* (which show long life spans for queens and workers, but have small colony sizes (Dammann and Burda 2006; Dammann et al. 2011)) without changing the results.

We found that worker and queen life span diverge (increasing life span ratio) with increasing colony size in eusocial Hymenoptera. This may point to a general trend in eusocial organisms, which is already visible at the transition from solitary to social species also for species not belonging to the Hymenoptera. Two examples are the wasps and the *Fukomys* species (mole-rats), in which worker life span remains at levels of the solitary ancestors while queen life span increases in the social species. In honey bees (*Apis mellifera*) the same pattern is observed throughout colony growth: in small colonies workers have longer lives when compared with workers living in larger colonies (Rueppell et al. 2009). Increasing colony size leads to a number of social consequences: the reproductive potential of workers decreases while the social complexity and the degree of caste differentiation increase (Alexander et al. 1991; Bourke 1999; Changizi et al. 2002). Life span as a life-history trait appears to be affected by those changes.

Our finding generally points to an adaptive process of division of labor and increasing task specialization that eusocial Hymenoptera have in common and that could lead to an adaptive demography, where the demography of the colony is directly adaptive and not its causal parameters. (Oster and Wilson 1978; Hölldobler and Wilson 1990). As colony size increases the quality of individuals diverges and life span as an outcome of maintenance investments may be evolutionary shaped to optimize productivity and hence fitness on the colony level by modifying the resource allocation into different castes. However, it is difficult to disentangle the effect of larger colony size on the life span differential. Colony size is associated with increasing division of labor, which in turn leads to morphological differences and different patterns of resource allocation into individuals possibly as a response to different levels of extrinsic mortality (Bourke 1999; Dornhaus et al. 2012; Muench et al. 2008; Amdam and Omholt 2002). Thus, it is complicated to identify exclusive explanatory factors for the observed life span differential.

Following the evolutionary theories of aging, maintenance investment could be shaped according to the differences in extrinsic mortality and thus for social insects it has been argued that extrinsic mortality explains the life span of queens, the differences between worker and queen life span, as well as life span differences between different worker castes (Heinze and Schrempf 2008; Keller and Genoud 1997; Chapuisat and Keller 2002). Following this, one possible explanation for the increasing life span ratio is that workers of larger colonies experience higher levels of extrinsic mortality while queens are more heavily protected by their colony. Lager colony sizes may force colonies to increase their foraging range and to defend more distant food patches. The distance each forager has to cover during a foraging trip increases and thus the risk of dying from predation (Jorgensen and Porter 1982). Additionally, competition with other colonies might increase for species with larger foraging ranges (Gordon and Kulig 1996). Selection may act on the colony level to ensure resources within the protected colony and minimize losses caused by the early death of a worker. Individual worker productivity should be optimized: applying a model of intergenerational transfers to honey bees suggests that task- or caste-dependent senescence patterns may occur as a result of different schedules of resource allocation to individuals dependent on the task carried out (Amdam and Page 2005). In larger colonies selection for morphological or physiological changes that increase worker productivity should be more efficiently selected for because the benefits would be multiplied by the number of workers. This trait could be selected for at the colony level to increase colony fitness by changing the life histories of the individuals within, resulting in disposable workers (Porter and Jorgensen 1981) and highly reproductive and long-lived queens. Additionally, redundancy and parallel operations leads to a reduced dependency of the superorganism on the precise functioning of single individuals (Oster and Wilson 1978). As long as the required tasks are accomplished and no fitness compromises are made, selection on worker live span might be weak, leading to workers that expend fewer resources independent of extrinsic mortality. It has been shown that small short-lived workers of *Solenopsis invicta* are less costly for the colony than large workers (Calabi and Porter 1989).

Alternatively the differences in life span could be explained by the morphological differences, but in our study we find that colony size and not the increasing morphological differences measured by the dry weight ratio explain the difference in life span between queens and workers. Even in species with morphologically identical castes, which differ in social or reproductive status, life span may differ. Examples are ant species with gamergate systems such as *Platythyrea punctata* or *Diacamma cf. rugosum* (Hartmann and Heinze 2003; Tsuji et al. 1996) or social wasps such as *Roplidia marginata* (Gadagkar et al. 1993), which are included in our dataset.

However, worker life span may be correlated with body size (Porter and Tschinkel 1985; Calabi and Porter 1989), independent of size as a response to the level of extrinsic mortality (Chapuisat and Keller 2002) or may be task-dependent (Rueppell et al. 2007), and a combination of those factors may ultimately explain the interspecific differences of worker life span as a response to colony size. Thus, in this study we cannot be certain which factors drive the positive association of colony size and life span.

In general we cannot be sure why the observed life span differences appear as a response to changes in colony size. But we can confirm a larger organismal trend, namely the increasing specialization with increasing size within the superorganism. As a general rule governing the evolution of organismal complexity larger entities (organisms, nervous systems, ecosystems and ant colonies (superorganisms)) are composed of increasing numbers of subunits (Changizi et al. 2002; Bell and Mooers 1997). In multicellular organisms division of labor measured as the number of cell types increases with body size (Bell and Mooers 1997), in ant colonies the number of castes increases with colony size (Changizi et al. 2002). Here we can show for the social insects that the increasing number of phenotypes also leads to an increasing divergence and specialization of those phenotypes. Although we can confirm the increasing morphological differences with colony size measured as weight (this study) or size differences (Changizi et al. 2002), we can also show that life-history traits may also be affected. The measured trait life span diverges as a response to colony size and increasing complexity.

Conclusions

For social Hymenoptera life span shows a strong association with colony size once the differences between workers and queens are taken into account. The queen-worker life span ratio (queen life span / worker life span) is positively associated with colony size, while the association between queen or worker life span with colony size is not existent or weak. If we assume that the differences in life span between queen and worker of a species arise from increasing task specialization in larger groups we find a general pattern in eusocial

Hymenoptera that confirms increasing task specialization of individuals, consistent with increasing organismal complexity found in the evolution of nervous systems, multicellular organisms, ant colonies and ecosystems (Changizi et al. 2002; Bell and Mooers 1997). In social insects, caste specialization and reproductive division of labor are often accompanied by different levels of extrinsic mortality, which may lead to the correlation of life span differences with increasing colony size. To elaborate further the link between extrinsic mortality it would be interesting to test if workers in larger colonies generally experience higher levels of extrinsic mortality.

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Figures and tables

Table 1: Results of the linear regressions of worker life span, queen life span and life span ratio (queen/worker) with colony size.

	Family	Intercept	P-value	Slope	P-value	R^2	DF
Worker	*Formicidae*	792.90	<0.001	-0.0001	0.3620	0.021	39
	Vespidae	76.21	0.002	-0.0057	0.5030	0.046	10
	Apidae	74.42	0.002	0.0015	0.4475	0.074	8
	Hymenoptera	523.10	<0.001	-0.0001	0.6140	0.004	64
Queen	*Formicidae*	3736.00	<0.001	0.0010	0.0392	0.070	59
	Vespidae	286.89	<0.001	0.0635	0.7445	0.014	8
	Apidae	698.62	0.139	0.0967	0.0539	0.761	3
	Hymenoptera	3026.00	<0.001	0.0012	0.0117	0.080	77
Queen/ Worker	*Formicidae*	-	-	0.2060	<0.001	0.898	30
	Vespidae	-	-	0.3220	<0.001	0.855	9
	Apidae	-	-	0.3290	0.0023	0.904	4
	Hymenoptera	-	-	0.2300	<0.001	0.867	48

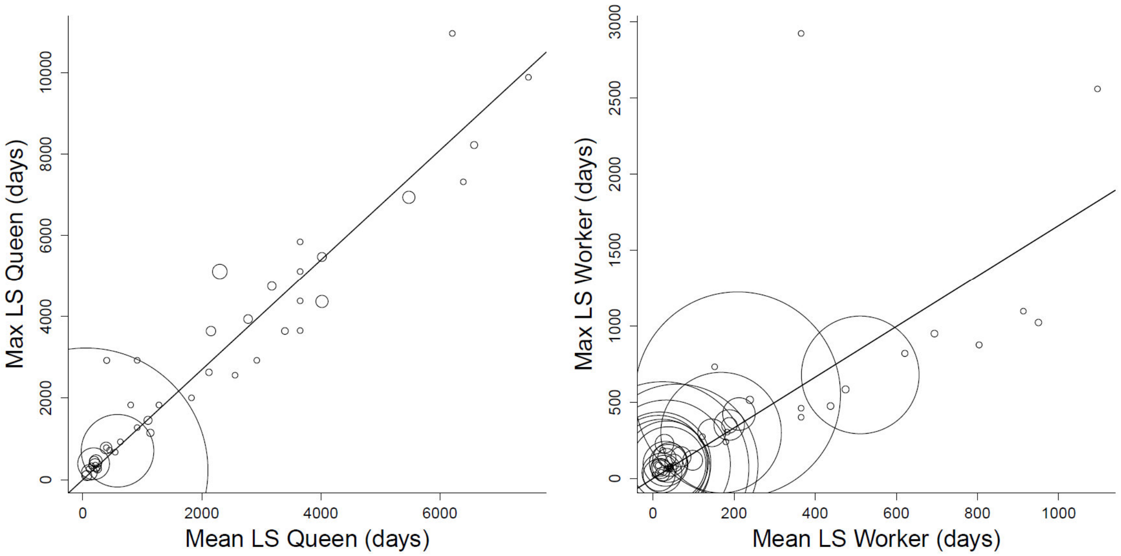

Figure 1: Linear regressions between mean and maximum life span for queens (left) and workers (right). Each circle represents mean and maximum life span of a species. The size of the circle represents sample size. The lines represent the slope (conversion factor) estimated from regression through the origin weighted by sample size. Left: mean and maximum queen life span; slope: 1.34±0.05 ($t_{1,44}$ = 28.32, P <0.001, R^2 = 0.95, F = 802.2). Right: mean and maximum worker life span; slope: 1.66±0.07 ($t_{1,55}$ = 21.15, P <0.001, R^2 = 0.89, F = 447.3).

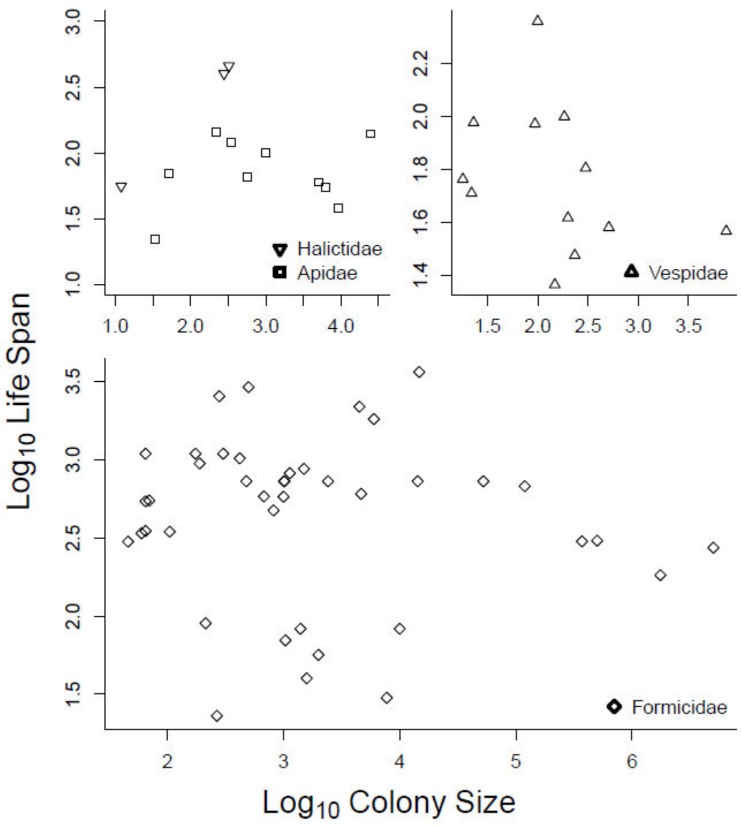

Figure 2: Maximum worker life span and group size. 66 species (Hymenoptera) are included in this plot (*Formicidae*= 41 species (intercept = 792.9, P <0.001, slope = -0.0001, P = 0.362, R² = 0.021, DF = 39, F = 0.85), *Vespidae*= 12 species (intercept = 76.21, P = 0.002, slope = -0.006, P = 0.503, R² = 0.046, DF = 10, F = 0.483), *Apidae*= 10 species (intercept = 74.42, P = 0.002, slope = 0.0015, P = 0.448, R² = 0.074, DF = 8, F = 0.638), *Halictidae*= 3 species).

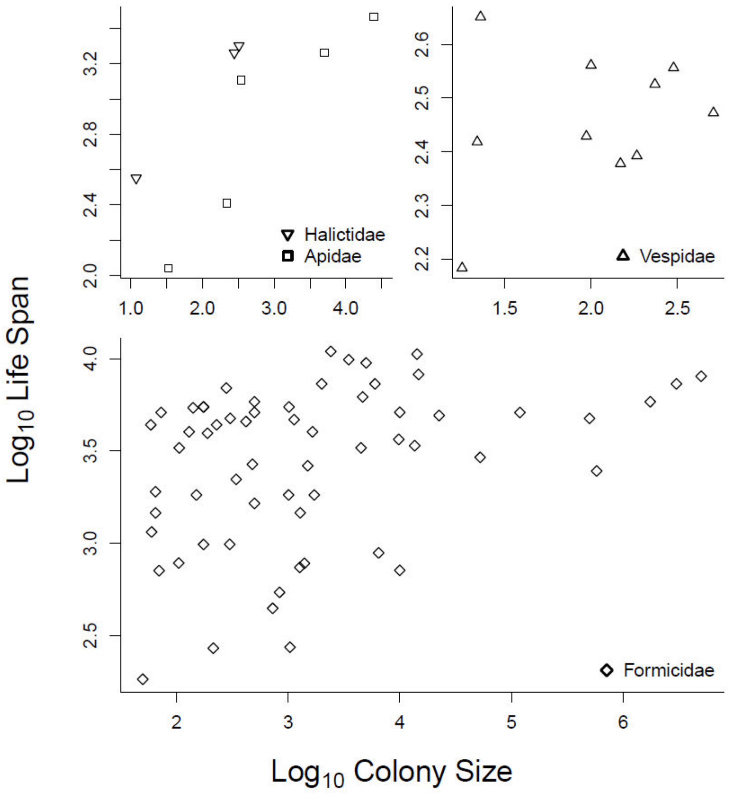

Figure 3: Maximum queen life span and colony size. 79 species (Hymenoptera) are included (*Formicidae*= 61 species (intercept = 3736, P <0.001, slope = 0.001, P = 0.039, R^2 = 0.07, DF = 59, F = 4.447), *Vespidae*= 10 species (intercept = 286.89, P <0.001, slope = 0.064, P = 0.745, R^2 = 0.014, DF = 8, F = 0.114), *Apidae* = 5 species (intercept = 698.62, P = 0.139, slope = 0.0967, P = 0.054, R^2 = 0.761, DF = 3, F = 9.528), *Halictidae*= 3 species).

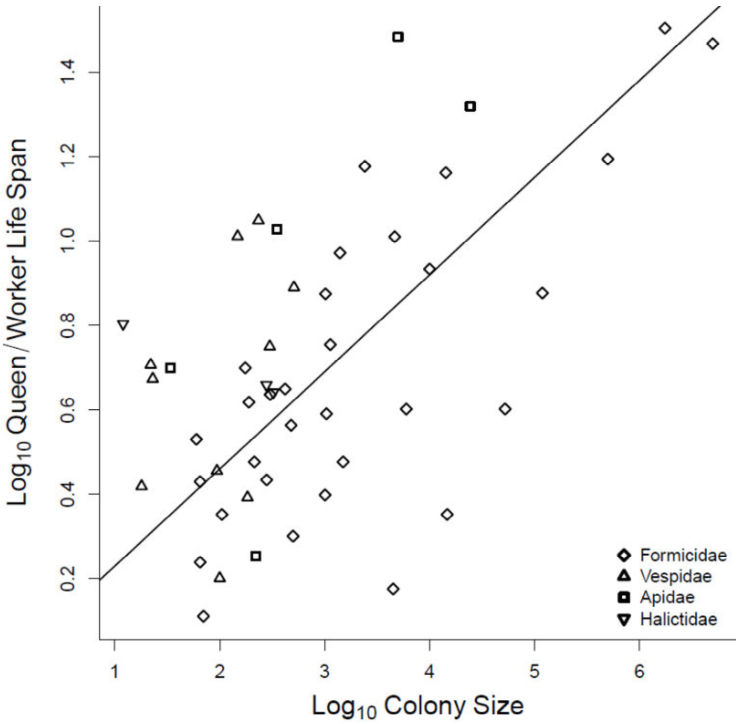

Figure 4: Maximum life span ratio and group size including 49 eusocial Hymenoptera species (*Formicidae*= 31 species, *Vespidae*= 10 species, *Apidae*= 5 species, *Halictidae*= 3 species). The line represents the results of the linear regression (slope= 0.23±0.01 ($t_{1,48}$= 17.14, P<0.001, R^2=0.86, F=293.7).

Supplemental material

Table S1: Species used in the independent contrast analysis.

Family	**Genus**	**Species**	**Code**
Apidae	*Apis*	*mellifera*	204
Apidae	*Bombus*	*terrestris*	215
Apidae	*Melipona*	*favosa*	221
Apidae	*Tetragonisca*	*angustula*	227
Formicidae	*Aphaenogaster*	*rudis*	237
Formicidae	*Atta*	*sexdens*	241
Formicidae	*Diacamma*	*sp.*	258
Formicidae	*Formica*	*sanguinea*	269
Formicidae	*Lasius*	*niger*	275
Formicidae	*Messor*	*semirufus*	278
Formicidae	*Monomorium*	*viride*	285
Formicidae	*Myrmecia*	*gulosa*	286
Formicidae	*Myrmica*	*rubra*	299
Formicidae	*Paraponera*	*clavata*	309
Formicidae	*Platythyrea*	*punctata*	311
Formicidae	*Solenopsis*	*invicta*	318
Formicidae	*Temnothorax*	*nylanderi*	325
Halictidae	*Halictus*	*ligatus*	334
Halictidae	*Lasioglossum*	*marginatum*	336
Vespidae	*Polistes*	*chinensis*	359
Vespidae	*Polistes*	*cinerascens*	360
Vespidae	*Polistes*	*fuscatus*	364
Vespidae	*Polistes*	*lanio*	367
Vespidae	*Polistes*	*versicolor*	369
Vespidae	*Ropalidia*	*marginata*	372
Vespidae	*Vespa*	*orientalis*	375
Vespidae	*Vespa*	*simillima*	376
Vespidae	*Vespa*	*tropica*	377

Table S2: Species list and used citations.

Genus	**Species**	**Reference**			
		Queen LS	**Worker LS**	**Colony size**	**Dry-weight**
Acromyrmex	*octospinosus*	(Weber 1972)	(Weber 1972)	(Weber 1972)	
Acromyrmex	*subterraneus*		(Camargo et al. 2007)	(Hölldobler and Wilson 1990)	
Aphaenogaster	*rudis*	(Haskins and Enzmann 1960)	(Hölldobler and Wilson 1990)	(Hölldobler and Wilson 1990)	(Trivers and Hare 1976; Lynch et al. 1980)
Apis	*cerana*		(Dyer and Seeley 1991)	(Seeley 1982)	
Apis	*florea*		(Dyer and Seeley 1991)	(Seeley 1982)	
Apis	*mellifera*	(Bozina 1961)	(Dyer and Seeley 1991; Ribbands 1952; Rueppell et al. 2005)	(Page and Peng 2000; Hou et al. 2010)	(DeGrandi-Hoffman et al. 2004; Hrassnigg and Crailsheim 2005; Page and Metcalf 1984)
Atta	*cephalotes*	(Weber 1972; Fjerdingstad and Boomsma 1998)	(Weber 1976)	(Beckers et al. 1989)	(Seal 2009; Shik 2010)
Atta	*colombica*	(Fjerdingstad and Boomsma 1998)	(Fowler et al. 1986)	(Martin et al. 1967)	(Shik 2010; Fjerdingstad and Boomsma 1997)
Atta	*sexdens*	(Weber 1972; Hölldobler and Wilson 1990)	(Fowler et al. 1986)	(Stradling and Powell 1986; Weber 1972)	
Bombus	*atratus*		(da Silva-Matos and Garofalo 2000)	(Gonzalez et al. 2004)	
Bombus	*diversus*	(Katayama 1996)		(Husband 1977)	
Bombus	*melanopygus*	(Owen and Plowright 1982)	(Cartar 1992)	(Cartar 1992)	
Bombus	*terrestris*	(Lopez-Vaamonde et al. 2009)	(Smeets and Duchateau 2003)	(Hou et al. 2010; Hasselrot 1960; Duchateau and Velthuis 1988)	(Duchateau et al. 1994; Duchateau and Velthuis 1988)
Camponotus	*fellah*	(Vonshak 2009)		(Vonshak 2009)	
Camponotus	*herculeanus*	(Hölldobler and Wilson 1990)		(Hou et al. 2010; Kaspari and Vargo 1995)	
Cardiocondyla	*emeryi*	(Keller 1998)		(King and Porter 2007)	
Cataglyphis	*bicolor*		(Schmid-Hempel and Schmid-Hempel 1984)	(Schmid-Hempel and Schmid-Hempel 1984; Beckers et al. 1989)	
Crematogaster	*ashmeadi*	(Hou et al. 2010)		(King and Porter 2007; Hou et al. 2010)	
Crematogaster	*scutellaris*	(Casevitzweulersse 1991)		(Depickere et al. 2008)	
Diacamma	*ceylonense*		(Tsuji et al. 1996)	(Monnin and Peeters 2008)	
Diacamma	*cyaneiventre*	(Andre et al. 2006)	(Andre et al. 2006)	(Andre et al. 2006)	

Genus	**Species**	**Reference**			
		Queen LS	**Worker LS**	**Colony size**	**Dry-weight**
Diacamma	*rugosum*	(Tsuji et al. 1996)	(Tsuji 1996)	(Hou et al. 2010; Hölldobler and Wilson 1990; Kaspari and Vargo 1995)	
Diacamma	*sp.*	(Tsuji et al. 1996)	(Tsuji 1996)	(Monnin and Peeters 2008)	
Ectatomma	*planidens*		(Antonialli et al. 2007)	(Antonialli et al. 2007)	
Ectatomma	*ruidum*	(Haskins and Haskins 1980)		(Hou et al. 2010; Lachaud et al. 1999)	
Formica	*exsecta*	(Pamilo 1991)		(Hou et al. 2010; Haag-Liautard et al. 2009; Hölldobler and Wilson 1990)	
Formica	*fusca*	(Keller 1998)	(Bierwirth 2003)	(Beckers et al. 1989; Seifert 2007)	(Jensen and Nielsen 1975)Keller pers. comm.
Formica	*polyctena*	(Hou et al. 2010)		(Seifert 2007; Beckers et al. 1989)	
Formica	*rufa*	(Buschinger 2004)		(Hölldobler and Wilson 1990; Kaspari and Vargo 1995)	
Formica	*rufibarbis*	(Hölldobler and Wilson 1990)		(Hou et al. 2010)	
Formica	*sanguinea*	(Hölldobler and Wilson 1990; Buschinger 2004)	(Bierwirth 2003)	(Seifert 2007)	
Formica	*selysi*	(Rosset and Chapuisat 2007)		(Hou et al. 2010; Rosset and Chapuisat 2007)	
Halictus	*ligatus*	(Richards and Packer 1995a)	(Richards and Packer 1995b)	(Boomsma and Eickwort 1993)	
Halictus	*marginatus*	(Plateaux-Quenu 1962)	(Plateaux-Quenu 1962)	(Hou et al. 2010)	
Harpagoxenus	*saltator*	(Peeters et al. 2000)	(Peeters et al. 2000)	(Hou et al. 2010)	
Harpagoxenus	*sublaevis*	(Keller 1998)		(Hou et al. 2010; Seifert 2007)	
Lasioglossum	*marginatum*	(Plateaux-Quenu 1962)	(Plateaux-Quenu 1962)	(Hou et al. 2010)	
Lasius	*alienus*	(Hölldobler and Wilson 1990)		(Hou et al. 2010; Brian 1978)	
Lasius	*flavus*	(Hölldobler and Wilson 1990)	(Schreiber 1969)	(Hou et al. 2010; Seifert 2007; Brian 1978)	(Keller and Passera 1989; Jensen and Nielsen 1975)Keller pers. comm.
Lasius	*niger*	(Hölldobler and Wilson 1990)	(Hölldobler and Wilson 1990)	(Hou et al. 2010; Fjerdingstad et al. 2003; Brian 1978)	(Fjerdingstad and Keller 2004; Keller and Passera 1989; Boomsma et al. 1995; Jensen and Nielsen 1975)
Leptothorax	*acervorum*	(Seifert 2007; Keller 1998)		(Seifert 2007; Beckers et al. 1989; Heinze and Ortius 1991)	

Genus	Species	Reference			
		Queen LS	**Worker LS**	**Colony size**	**Dry-weight**
Linepithema	*humile*	(Hou et al. 2010)		(Hou et al. 2010)	
Melipona	*beecheii*		(Biesmeijer and Toth 1998)	(Wenseleers et al. 2004)	
Melipona	*favosa*	(Koedam et al. 1995)	(Sommeijer 1984)	(Sommeijer 1984)	
Messor	*semirufus*	(Tohme and Tohme 1978)	(Tohme and Tohme 1978)	(Tohme and Tohme 1978; Hölldobler and Wilson 1990)	
Mischocyttarus	*cerberus*	(Giannotti and Machado 1994)	(Giannotti 1999)	(Harada Penna et al. 2007; Giannotti 1999)	
Monomorium	*minimum*	(DuBois 2000)	(DuBois 2000)	(Center et al. 2010)	
Monomorium	*pharaonis*	(Peacock and Baxter 1950)	(Peacock and Baxter 1950)	(Seifert 2007; Jackson et al. 2006; Brian 1978)	
Monomorium	*viride*	(DuBois 2000)	(DuBois 2000)	(King and Porter 2007)	
Myrmecia	*gulosa*	(Haskins and Haskins 1980)	(Haskins and Haskins 1980)	(Hou et al. 2010; Haskins and Haskins 1980; Beckers et al. 1989)	
Myrmecia	*nigriceps*	(Haskins and Haskins 1980)	(Haskins and Haskins 1980)	(van Wilgenburg et al. 2007)	
Myrmecia	*nigrocincta*		(Haskins and Haskins 1980)	(Kaspari and Vargo 1995)	
Myrmecia	*pilosula*		(Haskins and Haskins 1980)	(Kaspari and Vargo 1995)	
Myrmecia	*vindex*	(Haskins and Haskins 1980)	(Haskins and Haskins 1980)	(Hou et al. 2010; Haskins and Haskins 1950)	
Myrmecocystus	*mimicus*	(Hölldobler and Wilson 1990)		(Bartz and Holldobler 1982)	
Myrmica	*limanica*	(Elmes and Petal 1990)		(Elmes and Petal 1990)	
Myrmica	*lobicornis*	(Seppa 1994)		(Hou et al. 2010; Seppa 1994)	
Myrmica	*rubra*	(Seifert 2007)	(Hölldobler and Wilson 1990)	(Seifert 2007; Elmes 1974; Beckers et al. 1989)	(Fokuhl et al. 2007; Jensen and Nielsen 1975)
Myrmica	*ruginodis*	(Seppa 1994)		(Hou et al. 2010; Seppa 1994; Hölldobler and Wilson 1990; Brian 1950)	
Myrmica	*sabuleti*	(Brian 1972)		(Hou et al. 2010; Seifert 2007; Brian 1972)	
Myrmica	*sulcinodis*	(Hou et al. 2010)		(Hou et al. 2010; Elmes 1987)	
Myrmica	*tahoensis*	(Hou et al. 2010)		(Hou et al. 2010)	
Myrmicocrypta	*buenzlii*	(Weber 1972)		(Weber 1972)	
Odontomachus	*haematodus*	(Haskins and Haskins 1980)		(Hou et al. 2010; Beckers et al. 1989)	

Genus	Species	Reference			
		Queen LS	Worker LS	Colony size	Dry-weight
Oecophylla	*smaragdina*		(Chapuisat and Keller 2002)	(antARK 2012)	
Paraponera	*clavata*	(Zoo 2012)	(Zoo 2012)	(Janzen and Carroll 1983; Hölldobler and Wilson 1990)	
Pheidole	*dentata*		(Calabi and Traniello 1989)	(Calabi and Traniello 1989; Johnston and Wilson 1985)	
Platythyrea	*punctata*	(Hartmann and Heinze 2003)	(Hartmann and Heinze 2003)	(King and Porter 2007)	
Pogonomyrmex	*badius*	(Hölldobler and Wilson 1990)	(Tschinkel 1999)	(King and Porter 2007; Hou et al. 2010)	
Pogonomyrmex	*rugosus*		(Gordon and Holldobler 1987)	(Mackay 1981)	
Pogonomyrmex	*salinus*	(Porter and Jorgensen 1988)	(Porter and Jorgensen 1988)	(Porter and Jorgensen 1981)	
Polistes	*chinensis*	(Hou et al. 2010)	(Miyano 1980)	(Hou et al. 2010)	
Polistes	*cinerascens*	(Hou et al. 2010)	(Giannotti 1997)	(Hou et al. 2010)	(Kudo 2003)
Polistes	*fuscatus*	(West Eberhard 1969)	(Strassmann 1985)	(Hou et al. 2010)	
Polistes	*lanio*	(Giannotti and Machado 1994)	(Giannotti and Machado 1994)	(Hailey 2011)	
Polistes	*versicolor*	(Hou et al. 2010)	(Giannotti 1997)	(Hou et al. 2010)	
Polybia	*occidentalis*		(Odonnell and Jeanne 1992)	(Hurd et al. 2007)	
Rhytidoponera	*purpurea*	(Haskins and Haskins 1980)	(Haskins and Haskins 1980)	(Molet et al. 2008)	
Ropalidia	*marginata*	(Gadagkar et al. 1993)	(Gadagkar et al. 1982)	(Gadagkar et al. 1982)	(Sen and Gadagkar 2011)Gadagkar pers. comm..
Sericomyrmex	*urichi*	(Weber 1976)		(Weber 1972)	
Solenopsis	*invicta*	(Tschinkel 1987)	(Porter and Tschinkel 1985; Calabi and Porter 1989)	(Hou et al. 2010; Tschinkel 1988; Hölldobler and Wilson 1990)	(Tschinkel 1988; Keller and Ross 1993; Boomsma et al. 1995)
Temnothorax	*affinis*	(Seifert 2007; Buschinger 2004; Keller 1998)		(Seifert 2007)	
Temnothorax	*allardycei*		(Cole et al. 1994)	(Cole 1984)	
Temnothorax	*lichtensteini*	(Plateaux 1986)	(Hölldobler and Wilson 1990)	(Provost 1991)	Keller pers. comm..
Temnothorax	*nylanderi*	(Plateaux 1986)	S. Foitzik pers. comm..	(Beckers et al. 1989; Brian 1978)	(Foitzik and Heinze 2000; Pusch et al. 2006) Keller pers. Comm.
Temnothorax	*parvulus*	(Plateaux 1986)		(Seifert 2007)	
Temnothorax	*unifasciatus*	(Seifert 2007; Keller 1998)		(Beckers et al. 1989; Seifert 2007)	

Genus	**Species**	**Reference**			
		Queen LS	**Worker LS**	**Colony size**	**Dry-weight**
Tetragonisca	*angustula*	(Torres and Ruberson 2008)	(Torres et al. 2006)	(Hou et al. 2010)	
Trachymyrmex	*septentrionalis*	(Weber 1972)	(Beshers and Traniello 1996)	(King and Porter 2007; Weber 1972)	(Shik 2010; Seal 2009)
Trachymyrmex	*zeteki*	(Weber 1972)		(Weber 1972)	
Vespa	*orientalis*	(Oster 1976)	(Ishay et al. 1981) (Ishay et al. 1995)	(Hou et al. 2010	
Vespa	*simillima*	(Hou et al. 2010	(Matsuura 1984)	(Hou et al. 2010	
Vespa	*tropica*	(Hou et al. 2010	(Schmid-Hempel 1998)	(Hou et al. 2010	
Vespula	*vulgaris*		(Strassmann 1985)	(Burton and Burton 2002)	
Wasmannia	*auropunctata*	(Keller 1998)		(Hou et al. 2010)	

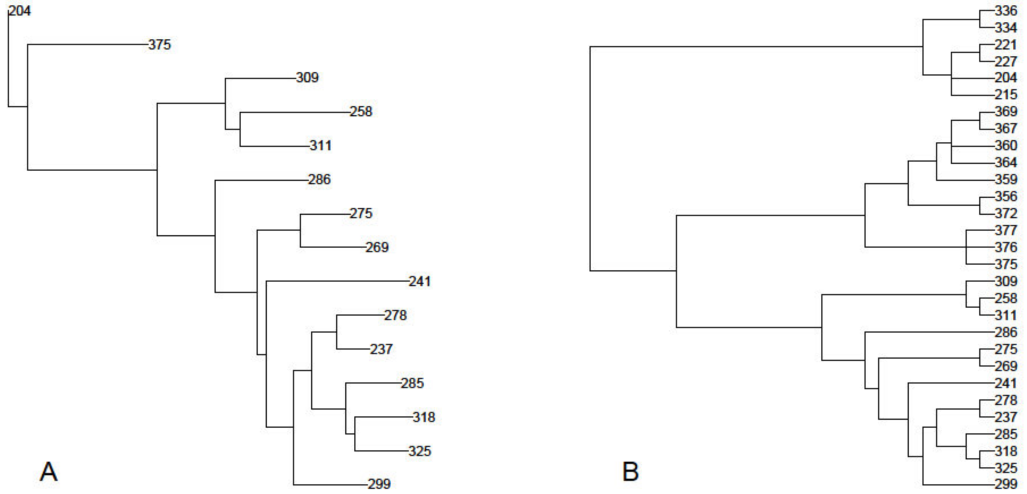

Figure S1: Phylogenetic trees used for the independent contrasts analysis. A) Inferred from (Moreau et al. 2006) including branch length. B) Inferred from (Arevalo et al. 2004; Moreau et al. 2006; Costa et al. 2003; Schmitz and Moritz 1998; Danforth 1999) using equal branch length.

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Chapter IV: Colony size and per-capita productivity in ants with single foragers

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Abstract

Optimal colony size in social insects is influenced by ecological and colony-intrinsic factors. An important intrinsic trait, the per-capita productivity, often declines in larger groups - a pattern known as "*Michener's paradox*". This phenomenon should pose serious problems to insect societies, and limit their growth. In this study, we first describe this problem, survey its prevalence across different ant species and then present a case study of eight cavity-dwelling ant species with very small colonies. In six out of these eight species, per-capita productivity indeed declined with size, so that the results match other studies on per-capita productivity in ants. We continue by offering mechanisms that can explain the decrease in per-capita productivity in our focal species. Their individual foraging mode leads to an increase in travel times with colony size for these central place foragers. We end by suggesting a potential strategy to overcome this limitation: the occupation of several nest sites at once (i.e., polydomy). We then show in one species that polydomy may help to circumvent the reduction in productivity with increasing size indicating that resource limitation causes the observed decrease in per-capita productivity. Finally, we discuss the influence of other factors such as the nesting ecology and colony homeostasis on the evolution of colony size in these cavity-dwelling ants.

Keywords: *colony size, Leptothorax, social insects, productivity, per-capita productivity, Michener effect, Temnothorax*

Introduction

Life-history evolution has shaped growth, reproduction and aging in animals (Stearns 1992). In social insects, colony size is a characteristic trait that forms the basis for various life-history trade-offs (Dornhaus et al. 2009; Bourke 1999). Similarly to body size in solitary organisms, it varies between populations and species. Some species of social insects reach enormous colony sizes of several million individuals (Beckers et al. 1989), whereas the colonies of other species (e.g., our focal species) contain only a few dozen individuals at maturity. Species-specific colony sizes are determined by factors both extrinsic (e.g., predation) and intrinsic (e.g., foraging mode) to the group (Jeanne and Nordheim 1996). The advantages of large colony size include better fighting abilities and defense, higher annual survival, and increased production of sexuals (Kaspari and Vargo 1995; Bourke 1999; Palmer 2004; Sorvari and Hakkarainen 2007; Wilson 1974; Cole 1984). On the other side, colony growth can be restricted by limited availability of construction materials or nesting sites, by a limited egg laying rate of the queen or due to resource limitation caused for example by the foraging mode of the species (Foitzik and Heinze 1998; Beckers et al. 1989; Wenzel and Pickering 1991).

An important intrinsic measure of colony fitness is the biomass production, which has been used to quantify the benefits of group living (Wcislo and Tierney 2009). Two common fitness measures in social insects, colony productivity and per-capita productivity, are defined here as the total number of new workers and sexuals produced by a colony and this productivity divided by the total number of workers (Clouse 2001; Scharf et al. 2011a). It was suggested that colonies should only grow larger if each additional group member leads to a per-capita fitness increase (Naug and Wenzel 2006). But, many eusocial species face a decline in per-capita productivity with increasing colony size or after exceeding a certain colony size

(Michener 1964; Cassill 2002; Litte 1981), a pattern known as the "Michener's paradox" (Michener 1964). Within the Formicidae studies on changes in per-capita productivity with colony size show that decreasing (Porter and Tschinkel 1985; Tschinkel 1993; Tschinkel 1999; Brian 1953, 1956; Cole 1984; Wilson 1974; Hee et al. 2000; Cassill 2002; Franks et al. 2006) and constant (Kaspari and Byrne 1995; Billick 2001; Cole 1984; Hee et al. 2000) patterns occur while increasing patterns where never detected (see Table 1 for per-capita productivity in ants). Species with large colony sizes such as *Myrmica rubra, Solenopsis invicta* and *Pogonomyrmex badius* show a peak in per-capita productivity followed by a decline in per-capita productivity at colony sizes much lower than the species-specific average (Brian 1953, 1956; Tschinkel 1999; Cassill 2002). These decreasing patterns of per-capita productivity have been linked to logistic population growth caused by resource limitation and increasing forager density or increasing numbers of inactive workers (Dornhaus et al. 2009; Porter and Tschinkel 1985; Naug and Wenzel 2006). Some species with small colony sizes such as *Pheidole* ants from the tropical litter (Kaspari and Byrne 1995) or *Temnothorax allardycei* (Cole 1984) exhibit no changes of growth rate or per-capita productivity with increasing colony size. For those species it was suggested that high levels of disturbances triggered by predators, parasites or by decomposing nesting sites keep colonies below their reproductive optimum and from reaching colony sizes at which resources limit colony growth (Cole 1984; Kaspari and Byrne 1995).

In this study, we investigate whether colony size related changes in per-capita and colony productivity can select for the small colony sizes found in cavity dwelling ants. Two mechanisms were suggested as being responsible for the small colony sizes of the cavity dwelling ants in this study. First, a decrease in per-capita productivity could select for small colonies (Michener 1964; Oster and Wilson 1978) and second, colonies may aim for larger colony sizes where productivity is higher but disturbances leading to worker and brood loss may prevent the cavity dwelling species from reaching larger colony sizes (Kaspari and Byrne 1995). Here we aim to test the hypothesis that ants with small colonies never reach sizes at which per-capita productivity is maximized. We focus on eight cavity-dwelling ant species from temperate forests with small colony sizes of the genus *Temnothorax*, *Myrmica Leptothorax* or related slavemaker species. If disturbances and not resource limitation lead to small colonies in these species, per-capita productivity is expected to stay constant or increase with size. Disturbances should primarily lead to worker and possibly additional brood loss and consequently both productivity and size of the colony are affected. Alternatively, a decline in productivity may point to a resource limitation as a cause for small colony size in these cavity dwelling ants. In contrast to the effect of disturbances, resource limitation should mainly affect the resource influx and consequently the productivity of the colony but not its size.

If we find decreasing per-capita productivity, we would hypothesize that the rising costs of central place foraging could lead to decreasing per-capita productivity in these solitary foraging species (Naug and Wenzel 2006). As a solution to overcome the costs of increasing travel time, large colonies may split to occupy several nesting sites to increase the foraging range of a colony. Polydomy, a characteristic trait of four of our study species (Stephens and Krebs 1986; Orians and Pearson 1979) could restore high per-capita productivity in large colonies, indicating a link between per-capita productivity, foraging mode and resource limitation. To test this we conducted a case study on the facultative polydomous and polygynous ant *T. longispinosus* and used genetic data to identify nests that constitute a polydomous colony. Then we compared how the per-capita productivity changed with size in grouped and non-grouped nests.

Methods

Study systems

Cavity-dwelling ants of the genera Temnothorax, Leptothorax and Myrmica are characterized by both a small body and colony size. On average, their colonies contain only a few dozen ants. They inhabit the leaf litter layer of temperate forests and reside in acorns, nuts, sticks, or any wooden structure providing protection for their young (Moglich 1978; Foitzik and Heinze 1998; Herbers and Johnson 2007). Competition for nest sites is a limiting factor in these ants, as nest sites quickly decompose, and therefore colonies often relocate their nests (Herbers 1986; Holldobler and Wilson 1990; Byrne 1994; Foitzik and Heinze 1998). Larger colonies usually occupy larger nest sites (Herbers 1986; Foitzik and Heinze 1998; Pratt and Pierce 2001; Scharf et al. 2011b), and the cavity size of the nest site is probably the most important limiting factor for colony size (Byrne 1994; Kaspari 1996). A possible solution to space limitation is polydomy, i.e., the occupation of multiple nest sites per colony. In addition, cavity-dwelling ants are often polygynous, which allows them to permanently split their colonies (Alloway 1979; Herbers 1986). They are food generalists which forage individually. They can, however, use a slow recruitment behavior called “tandem running” during nest movement, but never chemical or mass recruitment (Moglich 1978; Beckers et al. 1989; Herbers and Choiniere 1996).

Data collection and analyses

We analyzed 12 datasets on cavity-dwelling ants from four genera and eight species across six localities (see Table 2). The datasets include information on colony size (by counting the number of workers in each colony; mean values in Table 2, worker production (worker pupae and newly emerged workers) and sexual production (males, male pupae, new queens and queen pupae). Two slavemaking species are included in the dataset. Protomognathus americanus parasitizes Temnothorax species in North America and Harpagoxenus sublaevis parasitizes the Eurasian Leptothorax species. Since the sampling was destructive colony development could not be monitored over several seasons. The habitat of the different localities, temperate forest, has already been described in detail elsewhere: Abensberg (Abe.): (Scharf et al. 2011b); Sommerhausen South & West (So. W, So. S): (Foitzik et al. 2003); New York (NY) and West Virginia (WV):(Foitzik et al. 2009).

Colony productivity and per-capita productivity

We aimed here to understand how colony productivity and per-capita productivity are related to colony size. For the analysis we calculated the biomass (mg) of the colony productivity (workers, queens and males) using species-specific average biomass values for workers, queens and males (Table 2). The data for *Harpagoxenus sublaevis* is different since we just had information on the number of newly produced individuals, but no information on their caste. Hence, we used the number of produced individuals throughout our analysis.

First, we used the colony productivity and used linear regressions to determine the effect of colony size on productivity. To normalize the data we log-transformed both total production and colony size, which resulted in normally distributed residuals. The log transformation allows for easier interpretation of the relationship between size and productivity: A slope of 1 represents a constant increase in productivity with each added individual, a slope <1 represents decreasing marginal returns and a slope >1 represents increasing marginal returns.

To analyze whether next to colony size developmental stage (immature colonies: only worker production; mature colonies: sexual and worker production) was associated with productivity we conducted an ANCOVA, with colony size as the covariate, mature/immature colonies as the explanatory variable and productivity as the response variable. A significant interaction term (colony size × mature/immature colonies) would indicate different slopes in the pattern of per-capita productivity for mature and immature colonies. This analysis was not performed for *H. sublaevis* since we only had information on brood production.

To analyze the per-capita productivity we divided colony productivity by the number of workers present in the colony. In the case of the slavemaking species (P. americanus & H. sublaevis), we divided colony productivity by the number of slaves, because the slaves are conducting the workers' routine tasks and thus we expect these species to show similar result as the parasitized species (Alloway 1979; Pamminger et al. 2011).A major problem was that the variance of the dependent variable was not homogenous, but decreased with increasing colony size. The Breusch-Pangan test (Breusch and Pagan 1979) showed heteroscedasticity for most datasets (Table 4). To overcome this and to account for the variance heterogeneity in per-capita productivity, especially in small colonies we used quantile regressions (Koenker and Hallock 2001) to investigate the relationship between per-capita productivity and colony size. Quantile regression estimates multiple rates of change (slopes) from the minimum to the maximum response, providing a more complete picture of the relationships between variables (Cade and Noon 2003; Koenker and Bassett Jr 1978). Quantile regressions are semiparametric in the sense that no parametric distributional form is assumed for the error distribution (Cade 2003). From the quantile regression we received information about the best and worst performing colonies. The quantiles used (0.1, 0.25, 0.5, 0.75 and 0.9) represent the bottom 10% and 25%, the top 25% and 10% and the median (0.5) of the colonies in terms of per capita productivity. The differences in the quantile regression show the changes in variance over all colony sizes.

The effect of polydomy on per-capita productivity

In one dataset, T. longispinosus from New York, we tested whether polydomy could be used to overcome the Michener's paradox. We had data on the relatedness of T. longispinosus colonies from mapped 6×6m plots in NY (Foitzik et al. 2009). Up to three workers per colony (565 workers in 195 colonies) were genotyped at four microsatellite loci: LXA GT1, L-18, L-5 and Myrt3. Pair-wise relatedness value among all individuals in a plot was calculated using the Queller and Goodnight estimator of pair-wise relatedness (Queller and Goodnight 1989) within the program COANCESTRY (Wang 2011). To calculate the relatedness between two colonies, we used the average relatedness of all possible combinations between the individuals of the two colonies (e.g., if two ants of each colony are genotyped, then there are four possible combinations). Merging colonies based on genetic data is always problematic (Pedersen and Boomsma 1999), because it is unclear which cut-off point reflects real colony boundaries. In our study the median of the within-nest relatedness values was $r = 0.55$. Between-nest relatedness values had a median of 0.03, and only 3% of pair-wise nest comparisons had a higher relatedness value than 0.40. We then decided to merge colonies that were more closely related than $r = 0.40$, a value lower than the median relatedness within colonies but rarely reached in between colony comparisons. We only merged colonies that were less than 5m apart, which lies above the travel distance of foragers, and removed colonies with either no productivity or no workers before merging.

In addition to the general rules mentioned above, we applied two different schemes for merging. First, we merged all related nests independent of the number of queens (AR, all related). T. longispinosus is facultative polygynous in the New York population (Foitzik et al. 2009). We than followed the rule that each added nest must be related by >0.4 to two-thirds of the other merged nests. For the analysis we merged 89 nests to 38 colonies leaving 104 nests unmerged. We merged on average 2.3 (range 2-4) nests.

Since sub-nests are often used periodically and often contain no queen (Foitzik et al. 2009) we decided in a second approach to merge one queenright to several queenless nests if relatedness was >0.4 (later called OTM, or one to many). The more nests were merged, the more likely the between nest relatedness of the queenless nests showed a lower relatedness than 0.4. Therefore, we relaxed our relatedness criteria somewhat in-between queenless nests, which had to show a relatedness value above r >0.3. We merged 87 colonies from the original dataset to create 35 merged colonies leaving 110 colonies unmerged. The mean number of merged nests was 2.5 and we merged 2-6 nests.

We then tested for the change in per-capita productivity as a response to colony size between single and merged colonies. We used an ANCOVA, with colony size as the covariate, single/merged colonies as the explanatory variable and per-capita productivity as the response variable. A significant interaction term (colony size × single/merged colonies) would indicate different slopes in the pattern of per-capita productivity for merges and unmerged colonies.

To test for nest site limitation which might force bigger colonies to split we used the colonies from the same *T. longispinosus* dataset living in acorns (N=146). For acorns, as compared to sticks or other nesting cavities, a proportional relation between diameter and volume exists. We used linear regressions to test for a relationship between nest and acorn size measured as the acorn diameter.

All statistical analyses were performed using R-Statistical software (R Core Development Team 2010) and the package quantreg (Koenker 2011).

Results

Log-linear models

The log-linear models on colony productivity revealed that for most data sets and species (seven of 12 datasets and five of eight species) additional workers lead to decreasing marginal returns (slope ± confidence interval <1, Table 3, linear regression on colony size Fig 1). Change in productivity with size (measured as the slope ± confidence interval of the linear regressions) in both datasets on *T. crassispinus* included the slope of one, pointing to an independence of production and colony size. *T. crassispinus* was the only species where the slope estimate was >1. For the two populations of *P. americanus*, the confidence intervals around the slope estimate included one, but due to the small number of sampled colonies (N = 50 & N = 36) the confidence intervals are large compared to the other data sets (Table 3). We cannot rule out that colony productivity increases linearly for *P. americanus* (Fig. 1) with increasing size leading to constant per-capita productivity. The confidence interval on the slope for one *T. nylanderi* population (Sommerhausen West) also includes a slope of one, while the other population (Sommerhausen South) showed significant decreasing marginal returns (Table 3, Fig. 1). For all other species (*L. acervorum, L. muscorum, M. punctiventris, T. longispinosus* and *H. sublaevis*) and datasets colony productivity showed decreasing marginal returns, which can be interpreted as decreasing per-capita productivity (Table 3, Fig. 1).

The effect of sexual reproduction

For all populations we found that the sexual production occurred across all colony sizes. Next to colony size, developmental stage co-varied in three of the eleven populations with productivity. Mature colonies of *L. acervorum* Abensberg population (ANCOVA: $F_{4,156}$ =

324.9, P = 0.005), *T. nylanderi* Sommerhausen West population (ANCOVA: $F_{4,230} = 723.5$, P = 0.009) and *P. americanus* New York population (ANCOVA: $F_{4,31} = 71.66$, P = 0.008) showed higher productivity in colonies producing sexuals.

Quantile regressions and per-capita productivity

Quantile regressions allow a comparison of the subset of colonies that performed the best (upper quantiles) to the subset that performed the worst (lower quantiles) along different colony sizes. The quantile regression showed the potential effect of a decreasing variance in per capita productivity. At small sizes the expected productivity of the colony varies strongly while at lager sizes colonies are homogenous in their productivity. All datasets showed a switch from positive to negative slopes with increasing quantiles used for the regression analysis (Fig. 2, Table 4). When focusing on very productive colonies (quantiles: 0.9, 0.75), per-capita productivity decreased with colony size, while the opposite was true for the poorly performing colonies (quantiles: 0.1, 0.25), resulting in similar per-capita productivity in larger colonies. Further, there was a decreasing variance in per-capita productivity with increasing colony size as indicated by the quantile regressions and the Breusch-Pagan test (Table 4). In all datasets decreasing variance can be seen in the different intercepts and the convergence of the quantile regressions (Fig. 2, Table 4). For some species the quantile regressions overlapped at large colony sizes: *T. nylanderi* in both populations, *T. longispinosus* in both populations, *M. punctiventris* and *T. crassispinus* in the Regensburg population. The other populations show the same trend, but convergence if the regression lies outside of the colony size rage of the population (Fig. 2). As in the linear models on log transformed data *T. crassispinus* did not show a decrease in per-capita productivity with increasing size. Per-capita productivity was either constant (Abensburg population) or increasing (Regensburg population). Only the 0.9 quantiles showed a negative slope. Interestingly, the two *P.*

americanus populations showed opposite trends (Table 4, Fig. 2). For the NY population the median quantile (0.5) estimate showed increasing per-capita productivity, while for the WV population a decrease in per-capita productivity was found. *L. acervorum, H. sublaevis, M. punctiventris* and one of the *T. nylanderi* populations (Sommerhausen west) showed slightly positive per-capita productivity in the median (0.5) quantile (Table 4). Decreasing per-capita productivity was found for *T. nylanderi* (Sommerhausen south), *T. longispinosus* (both populations), *P. americanus* (West Virginia) and *L. muscorum* (Table 4, Fig. 2).

A possible solution: the effect of polydomy on productivity

T. longispinosus colonies from the NY dataset living in acorns showed a significant relation between colony size and the diameter of the acorn housing those colonies. The slope of the linear regression (intercept: 14.55, $p<0.001$; slope: 0.03, $P=0.006$; $R^2=0.04$; $F=7.77$; DF= 145) is significant, but close to zero pointing to a weak relationship between colony size and acorn diameter. Due to the relation of diameter and volume of spherical objects we would have expected a stronger increase of worker in larger acorns if these are limited.

The association of per-capita productivity and colony size differed between merged vs. unmerged colonies as indicated by the significant interaction term (colony size x merged/unmerged colonies) in the OTM method (ANCOVA: $F_{1,110} = 6.450$, $P = 0.012$) that point to different slopes for both groups meaning that the decline in productivity with colony size is attenuated in polydomous colonies. This interaction was not significant for the AR approach (ANCOVA $F_{1,110} = 1.853$, $P = 0.176$; Fig. 3).

Discussion

The decrease in per-capita productivity with colony size is predominant in ant societies (Table 1). The suggested mechanism behind this decline is the increasing number of foragers that leads to a depletion of resources and consequently to logistic population growth (Naug and Wenzel 2006). Our *Temnorthorax* and related genera case studies showed that this decrease in per-capita productivity is common in species with small colonies, especially when referring to the upper quartiles of the data (Fig.2, Table 4). To overcome this problem of limited foraging area, we suggest that colonies use polydomy, the occupation of more than one nest. Comparison of per-capita productivity of single and polydomous colonies partially supports this suggestion.

Our study weakens the hypothesis that disturbances prevent species with small colony sizes from reaching optimal productivity. If disturbances forestall the cavity dwelling species from reaching colony sizes where per-capita productivity is optimal we would have expected constant or increasing patterns of per- capita productivity. The log-linear models revealed that none of our study species showed increasing per-capita productivity and just one of our study species (*T. crassispinus*) showed constant patterns of per-capita productivity. For two species, *P. americanus* (both populations) and *T. nylanderi* (So. W. population), the confidence intervals of the log-linear model included constant per-capita productivity. It is therefore possible that the constant patterns result from the small colony size of these species as shown for *Pheidole* ants (Kaspari and Byrne 1995) and *Temnothorax allardycei* (Cole 1984), these colonies may never reach colony sizes where the surrounding resources are exploited (Cole 1984; Kaspari and Byrne 1995). But it remains an unanswered question why *T. crassispinus* clearly showed constant patterns while the closely related sister species *T. nylanderi* did not.

Most of our study species as well as the majority of other ant species (Table 1) showed declining patterns of per capita productivity. It was suggested that the onset of decline in per-capita productivity should occur at optimal sizes of the colony (Michener 1964; Oster and Wilson 1978). For none of the cavity dwelling species of this study we found a peak in productivity which could point to an optimal colony size with respect to productivity. In fact, for most of the cavity dwelling ants of this study reach the highest levels of productivity at very small colony sizes. Other studies showed that per-capita productivity decreased after colonies reached a size threshold but for *Myrmica rubra, Solenopsis invicta* and *Pogonomyrmex badius* this threshold is already reached at colony sizes lower than the species-specific average (Tschinkel 1999; Cassill 2002; Brian 1953, 1956; Porter and Tschinkel 1985; Tschinkel 1993). Sexual reproduction occurred at all colony sizes starting from colony sizes below 10 individuals for all datasets and more productive colonies had a higher chance of producing sexuals.

Declining patterns of per-capita productivity have been linked to logistic population growth, either caused by a limited egg laying rate of the queen as found for some wasps or other annuals species, by resource limitation or by the increasing number of inactive workers in larger colonies (Robinson 1992; Dornhaus et al. 2009; Porter and Tschinkel 1985; Jeanne and Nordheim 1996; Naug and Wenzel 2006). Indeed, larger colonies may be labor-saturated and keep a larger reserve work force indicated by increasing numbers of inactive workers (Mirenda and Vinson 1981; Robinson 1992; Dornhaus et al. 2009; Waters et al. 2010; Oster and Wilson 1978). However, it remains unclear whether the reserve work force is a response to the decrease in per-capita productivity caused by decreasing foraging returns or whether the productivity decline is caused by the increase in the number of inactive workers. We suggest that if additional workers add negligible productivity to the colony due to resource limitation, it may be beneficial for the colony to keep those workers inside the protected colony instead of putting them at risk to die in outside activities. Consequently, these reserve workers represent redundancy and ensure the system reliability of the colony (Oster and Wilson 1978; Porter and Tschinkel 1985).

Resource limitation may be caused by the fact that most social insect colonies are central-place foragers and thus resemble in some ways sessile organisms. As the colony grows, an increasing forager density lead to longer individual foraging trips as resources are exploited (Jorgensen and Porter 1982; Jun et al. 2003), and hence to a reduction in individual foraging returns. In fact, a theoretical approach using foraging economics showed that under certain conditions, more workers cannot lead to a further increase in productivity (Naug and Wenzel 2006), because density dependent effects lead to logistic population growth as shown for *Oecophylla smaragdina* (Gupta 1968), *Solenopsis invicta* (Tschinkel 1993) and *Pogonomyrmex occidentalis* (Wiernasz and Cole 1995) and may put an ultimate limit to colony productivity (Sudd 1987; Naug and Wenzel 2006; Michener 1964; Oster and Wilson 1978).

The individual foraging mode without recruitment as displayed by our focal species limits the foraging range, the number of foragers needed to efficiently exploit resources and the possibility to retrieve distant food items, leading to density-dependent reduction of foraging returns (Beckers et al. 1989). The optimal number of foragers should thus be smaller as compared to species with more complex foraging strategies (Beckers et al. 1989). A switch in the foraging strategy, or the ability to overcome larger prey items with increasing numbers of foragers as shown for social spiders (Yip et al. 2008; Pasquet and Krafft 1992) might circumvent the resource limitation and attenuate the decrease in per-capita productivity. It would be therefore interesting to see how the productivity changes with colony size in species such as *Lasius niger* that show a switch in the foraging strategy from individual to cooperative foraging as the colony grows (Fjerdingstad et al. 2003; Mailleux et al. 2003).

For species that did not evolve a more complex foraging mode an alternative solution to circumvent the decrease in per capita productivity may be to expand the foraging rage of a colony by splitting the colony to several nests in order to decrease travel time and worker density. Our analysis on the effect of polydomy on per-capita productivity indicated that a polydomous colony of *T. longispinosus* with a certain number of workers would perform better in terms of productivity than a monodomous colony with the same number of workers. Even if just one of the merged nests subset is performing significantly better that the non-merged / single nests, an increase in per-capita productivity by splitting the colony could be selected for to increase productivity. Sample sizes, especially for the merged colonies were low (35& 38 colonies), as a consequence the regression lines show large confidence intervals (Fig. 3).

Selection should act to increase the fitness of colonies, but the benefits on social living in terms of per-capita productivity are rarely found in insect societies. The only evidence for increasing patterns of per capita productivity is found within basal eusocial species such as *Bombus americanum*, *Polistes dominulus* and *Lasioglossum malachurum* (Michener 1964; Strohm and Bordon-Hauser 2003; Tibbetts and Reeve 2003).This indicates that per-capita productivity may be important during the evolution of eusociality (Cole 1984) but less important once eusociality and reproductive division of labor are established. In derived species such as ants per-capita productivity patterns (table 1) do not confirm that the evolution of colony size is solely driven by the productivity of the colony as suggested earlier (Michener 1964; Oster and Wilson 1978), but show that per-capita productivity, may only be a minor force in shaping species specific colony size (Jeanne and Nordheim 1996; Michener 1964). Therefore it is important to examine selective factors promoting larger colonies and their fitness benefits even if they generate costs in terms of per-capita productivity (Jeanne and Nordheim 1996; Michener 1964).

It is possible that colonies aim to reach a size at which colony homeostasis is high, production is predictable while ecological conditions do not moderate productivity of colonies as strongly as in smaller colonies (Naug and Wenzel 2006; Tindo et al. 2008). The quantile regression approach applied to our focal species suggests a trade-off between colony homeostasis and per-capita productivity (Fig. 2). Regressions on high and low performing colonies converged at the extremes of the colony size distributions caused by a high variance in per-capita production in small colonies as found in other studies (Stevens et al. 2007; Wenzel and Pickering 1991; Karsai and Wenzel 1998; Bouwma et al. 2006). The high variance in per-capita productivity at small colony sizes could be an outcome of disturbances caused by predators, environmental disturbances or variability in foraging success and the limited ability to buffer those extrinsic factors (Bouwma et al. 2006). The decrease in variance at larger colony sizes points to a reduced deviance from the expected food income or to increased foraging success predictability for the colony, and potentially selects for larger colony sizes (Stevens et al. 2007; Naug and Wenzel 2006; Wenzel and Pickering 1991; Kaspari and Byrne 1995; Kaspari and Vargo 1995).

Apart from improved colony homeostasis, increased colony size improves colony survival and thus potential future reproduction (Hou et al. 2010; Kaspari and Vargo 1995; Bourke 1999; Wilson 1974; Cole 1984). Cole (2009) has shown that for *Pogonomyrmex occidentalis* that increasing current reproduction at the expense of future reproduction may not be an advantageous strategy to maximize the reproductive value. Since per-capita productivity and fitness are tightly linked, increased survival promoted by larger colony sizes (Cole 1984; Wilson 1974), which leads to a decline in per-capita productivity, could be selected for to increase future productivity and subsequently the lifetime reproductive success and colony fitness.

Ultimately the small colony sizes of the cavity dwelling ant in this study may be explained by their nesting ecology. Cavity-dwelling species depend on naturally occurring cavities used as nesting sites, which are limited in size and availability. Wooden cavities decompose swiftly, forcing colonies to relocate to new nest sites regularly often leading to worker, queen or brood loss (Foitzik and Heinze 1998), which in turn limits colony growth and productivity. One strategy to overcome nest site limitation and to gain larger colonies is polydomy, a trait displayed by four of our study species (Table 2). Colonies may be forced to split their colonies because no adequate nesting site is available. Thus selection for polydomy would serve two functions, first decrease the dependency on large naturally occurring nesting sites and second to improve colony productivity which could otherwise be achieved by evolving more complex foraging modes. Seasonal polydomy as shown for *M. punctiventris* (Snyder and Herbers 2004) and several *Temnothorax* and *Leptothorax* species (Bourke and Franks 1995; Linksvayer 2008; Strätz and Heinze 2004; Foitzik and Heinze 2001) could be a strategy to increase colony efficiency and decrease the chance of being parasitized in summer, as larger colonies are more prone to an attack by slavemaking species (Pohl and Foitzik 2011), while increased colony size in winter could improve survival (Kaspari and Vargo 1995). Measuring long-term survival rates of cavity dwelling ant colonies is difficult, but may help to understand how survival limits colony size. Short-term data already suggest low survival rates, with only 33-50% of *Temnothorax* colonies surviving winter (Herbers and Johnson 2007), compared to much higher annual survival rates for mound-building ants of the *Formica rufa* group (68-92%), which also have larger colony sizes (Klimetzek 1981).

In conclusion, all ant species show declining or constant per-capita productivity. In contrast to earlier hypotheses we showed that even within species that form small sized colonies per-capita productivity may decline. Further our study showed that species specific colony size is not only predicted by optimal per-capita productivity, but that a number of factors promote small colony size while others promote larger colony sizes. Ecological factors (nest-site limitation) and social limitations (e.g., foraging mode (Beckers et al. 1989), which lead to declining per-capita productivity may limit colony size while factors such as colony homeostasis and increased survival probabilities of larger colonies should promote the evolution of larger colonies. Even though per-capita productivity may not be the most important factor to explain the colony size of a given species it might still put ultimate limits to colony growth and in combination with the naturally occurring nesting cavities explain why the cavity dwelling species did not evolve larger colony sizes. All species in this study have comparable life histories and since the slavemaking species depend on foragers from their host we did not expect differences in foraging ecology or resource influx. Thus it is puzzling that both constant and decreasing per-capita productivity were detected in our analyses. Anyway, polydomy may increase per-capita productivity, by extending the foraging area for species with central place foraging.

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Figures and tables

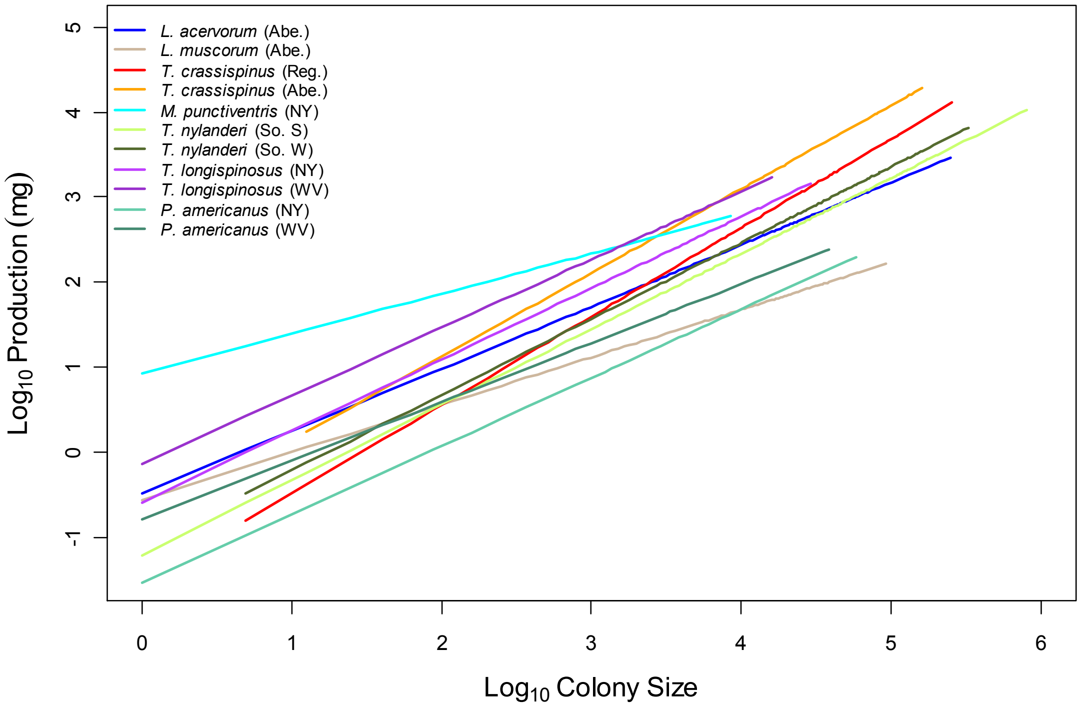

Fig. 1 Results of log-linear models showing the relationship between colony productivity and colony size (see Table 3 for the regression results)

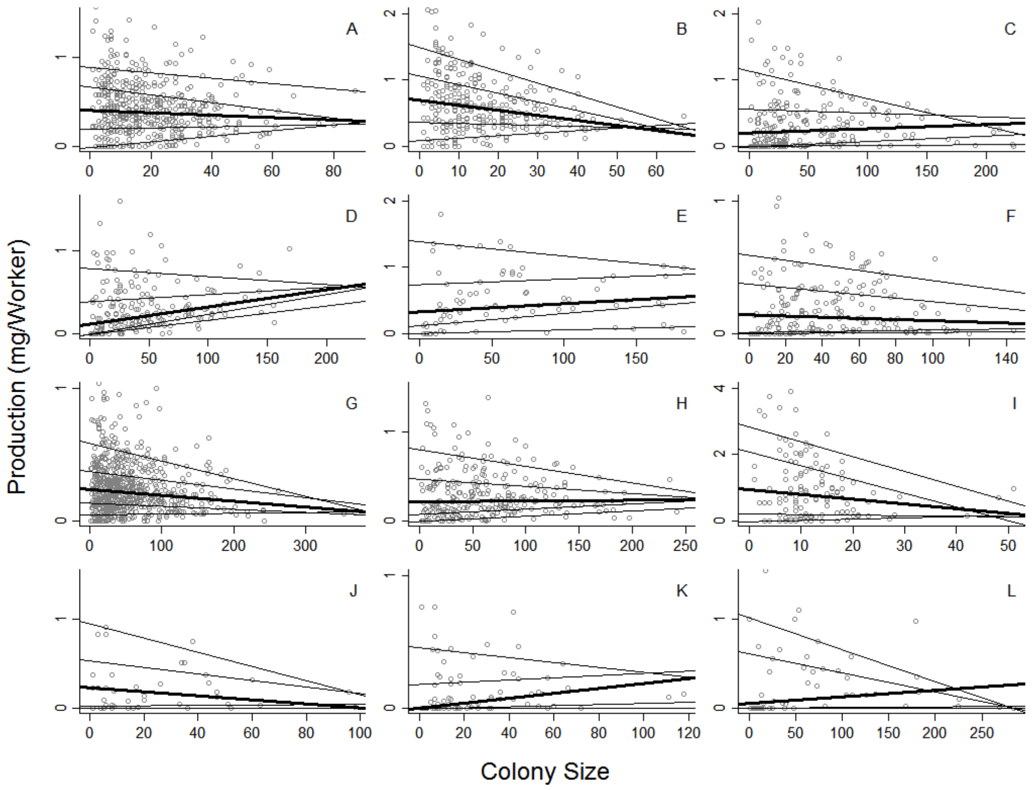

Fig. 2 Quantile regressions: The lines represent the results of the quantile regressions using different percentiles of the datasets (0.1; 0.25; 0.5; 0.75; 0.9). The 0.5 percentile is represented by the wider line. A: *T. longispinosus* (NY); B: *T. longispinosus* (WV); C: *L. acervorum* (Abe.); D: *T. crassispinus* (Reg.); E: *T. crassispinus* (Abe.); F: *L. muscorum* (Abe.); G: *T. nylanderi* (So. S.); H: *T. nylanderi* (So. W.); I: *M. punctiventris* (NY); J: *P. americanus* (WV); K: *P. americanus* (NY); L: *H. sublaevis* (Abe.). Note: for *H. sublaevis* the y axis represents individuals not mg/worker

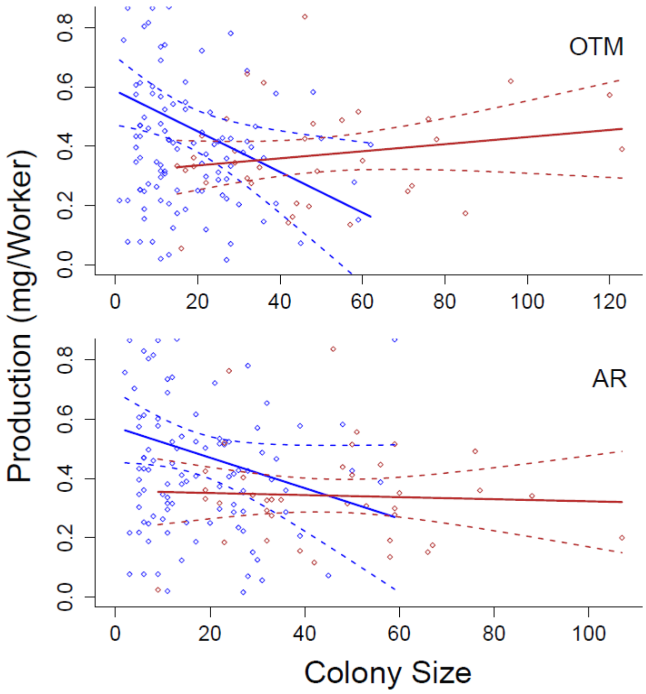

Fig. 3 Per-capita productivity (mg/worker) of *T. longispinosus* colonies in relation to colony size for the different merging strategies (OTM: one queenright nest and several queenless nests, AR: all related colonies). Single colonies are shown in blue and merged colonies in red. Solid line: linear regression; broken line: 95% confidence intervals.

Table 1: Per-capita productivity in ants.

Species	Study colony size	Decline at size	Productivity measure[2]	Response to colony size	Reference
Formica neorufibarbis	100-21000	-	p	constant	(Billick 2001)
Harpagoxenus sublaevis	1-250	-	o	decrease	this study
Leptothorax allardycei	1-100	-	ee	constant	(Cole 1984)
Leptothorax acervorum	1-220	-	bio	decrease	this study
Leptothorax muscorum	1-140	-	bio	decrease	this study
Temnothorax crassispinus	1-210	-	bio	constant	this study
Temnothorax curvispinosus	1-120	1	e+l	decrease	(Wilson 1974; Cole 1984)
Temnothorax longispinosus	1-90	-	bio	decrease	this study
Temnothorax nylanderi	1-330	-	bio	constant/ decrease[1]	this study
Linepithema humile	10-1000	100	b	decrease	(Hee et al. 2000)
Linepithema humile	1000-11000	-	b	constant	(Hee et al. 2000)
Myrmica punctiventris	1-50	-	bio	decrease	this study
Myrmica rubra	5-320	10	brc	decrease	(Brian 1953, 1956)
Pheidole	1-250	-	p	constant	(Kaspari and Byrne 1995)
Pogonomyrmex badius	100-10000	60	ee	decrease	(Tschinkel 1999)
Protomognatus americanus	1-120	-	bio	constant/ decrease	this study
Solenopsis invicta	100-300000	100, 0.75g	ee,sl,sp,o,bi o	decrease	(Porter and Tschinkel 1985; Tschinkel 1993; Cassill 2002)
Temnothorax albipennis	400	-	b	decrease	(Franks et al. 2006)

[1]: findings from different populations

[2]: ee=ergonomic efficiency (brood/worker); sl= sexuals larvae; sp= sexuals pupae, b=brood; sp= sexual production; p=pupae; bio= biomass; o=number of offspring; brc=brood rearing capability

Table 2: Study species information, sample sizes, mean colony size and weight data.

Dataset/ location	Density (nests / m2)	Monogynous (M) / Polygynous (P)	Monodomous (M) / Poldydomous (P)	Reference species	Sampled Colonies (N)	Colony Size (mean ,sd)	Dry mass Individual[5]	mg	Reference dry mass
L. acer. (Abe.) [6]	0.06	Facult. P	M	(Chan et al. 1999)	237	44.1 ±45.3	Q M W	0.412 0.432 0.401	[2]
L. musc. (Abe.) [6]	0.05	Facult. P	M	(Stuart and Alloway 1988)	204	40.3 ±31.0	Q M W	0.276 0.302 0.206	[2]
T. cras. (Abe.)	0.02	M	P	(Strätz and Heinze 2004)	83	58.6 ±53.3	Q[3] M[3]	0.921 0.19	(Strätz and Heinze 2004)
T. cras. (Reg.)	No data	M	P		198	43.5 ±38.2	W[3]	0.203	
T. nyla. (So. S)	7	M	P	(Foitzik and Heinze 1998; Foitzik et al. 2003)	730	51.4 ±47.5	Q M	0.921 0.19	(Foitzik and Heinze 2000)
T. nyla. (So. W)	3	M	P		273	60.5 ±50.1	W[4]	0.203	
T. long. (NY) [S]	0.8	Facult. P	P	(Herbers 1986)	498	18.2 ±14.3	Q	0.873	(Foitzik et al. 2004)
T. long. (WV) [S]	0.4	M	P	(Herbers and Stuart 1996)	295	15.1 ± 11.6	M W	0.231 0.217	
M. punc. (NY)	0.2	Facult. P	M	(DeHeer et al. 2001)[1]	134	12.7 ± 8.24	Q M W	1.16 0.63 0.55	(Herbers and Banschbach 1999)
P. amer. (NY)	0.1	M	P	(Foitzik and Herbers 2001)	83	3.06 ±5.61	Q M	0.58 0.19	(Herbers and Stuart 1998), this study
P. amer. (WV)	No data	M	P		49	5.95 ±7.42	W[4]	0.231	
H. subl. (Abe.)	0.01	M	M[2]	(Scharf et al. 2011a)	59	11.9 ±17.96			

[1] Differences among localities and years are evident and documented.

[2] Personal observations (B. Fischer-Blass and S. Foitzik).

[3] *T. crassispinus* dry mass is very similar to its relative *T. nylanderi*, and the dry mass of the latter has been already used in *T. crassispinus* studies (Strätz and Heinze 2004).

[4] *P. americanus* workers were weighted for this study (mean of 10 workers).

[5] W= Worker; Q=Queen; M=Male

[6] Parasitized by slavemaking ants.

Table 3: Relationship between colony size and colony productivity. Linear model on log transformed values.

Dataset	Intercept estimate	Pr(>|t|)	Slope estimate	Pr(>|t|)	R²	DF	Slope confidence intervals	
							0.025	0.975
L. acervorum (Abe.)	-0.486	0.187	0.731	0.000	0.233	181	0.537	0.926
L. muscorum (Abe.)	-0.561	0.190	0.558	0.000	0.119	169	0.328	0.789
T. crassispinus (Reg.)	-1.529	0.000	1.042	0.000	0.549	168	0.898	1.185
T. crassispinus (Abe.)	-0.849	0.083	0.985	0.000	0.468	72	0.738	1.232
M. punctiventris (NY)	0.922	0.013	0.470	0.002	0.080	117	0.178	0.762
T. nylanderi (So.S)	-1.218	0.000	0.887	0.000	0.494	697	0.821	0.954
T. nylanderi (So.W)	-1.114	0.000	0.893	0.000	0.435	247	0.766	1.021
T. longispinosus (NY)	-0.595	0.000	0.841	0.000	0.430	450	0.751	0.931
T. longispinosus (WV)	-0.139	0.333	0.801	0.000	0.433	278	0.693	0.909
P. americanus (NY)	-1.538	0.006	0.804	0.000	0.312	49	0.461	1.147
P. americanus (WV)	-0.8	0.135	0.694	0.000	0.297	35	0.327	1.061
H. sublaevis(Abe.)	0.31	0.688	0.567	0.004	0.213	35	0.193	0.940

Table 4: Results of the quantile regressions on per-capita productivity and the Breusch-Pagan test for heteroscedasticity.

Species & Location	Quantile	Intercept	p-Value	Slope	p-Value
L. acervorum (Abe.)	0.1	-0.001	<0.005	0	0.089
	0.25	-0.002	0.929	0.001	0.053
	0.5	0.198	0	0.001	0.321
	0.75	0.561	<0.005	-0.001	0.598
	0.9	1.14	<0.005	-0.004	<0.005
	Breusch-Pagan test			<0.005	
L. muscorum (Abe.)	0.1	-0.001	0.327	0	0.004
	0.25	0.008	0.379	0	0.063
	0.5	0.139	0	0	0.504
	0.75	0.372	<0.005	-0.001	0.121
	0.9	0.592	<0.005	-0.002	0.154
	Breusch-Pagan test			<0.005	
T. crassispinus (Reg.)	0.1	-0.01	0.086	0.002	<0.005
	0.25	-0.002	0.842	0.002	<0.005
	0.5	0.111	0.001	0.002	<0.005
	0.75	0.388	<0.005	0.001	0.397
	0.9	0.784	<0.005	-0.001	0.638
	Breusch-Pagan test			<0.005	
T. crassispinus (Abe.)	0.1	-0.002	0.914	0.001	0.096
	0.25	0.121	0.107	0.002	0.081
	0.5	0.336	0	0.001	0.256
	0.75	0.732	0	0.001	0.55
	0.9	1.382	0.009	-0.002	0.51
	Breusch-Pagan test			0.01	
H.sublaevis (Abe.)	0.1	-	-	-	-
	0.25	-0.001	0.875	0	0.778
	0.5	0.05	0.568	0.001	0.036
	0.75	0.614	0.002	-0.002	0.25
	0.9	1.023	0.088	-0.004	0.383
	Breusch-Pagan test			0.02	
M. punctiventris (NY)	0.1	-0.002	0.914	0.001	0.096
	0.25	0.121	0.107	0.002	0.081
	0.5	0.336	0	0.001	0.256
	0.75	0.732	0	0.001	0.55
	0.9	1.382	0.009	-0.002	0.51
	Breusch-Pagan test			<0.005	
P. americanus (NY)	0.1	-	-	-	-
	0.25	-0.003	<0.005	0	0.068
	0.5	0.001	0.985	0.002	<0.005
	0.75	0.177	0.014	0.001	0.293
	0.9	0.454	<0.005	-0.002	0.669
	Breusch-Pagan test			0.1	

Species & Location	Quantile	Intercept	p-Value	Slope	p-Value
P. americanus (WV)	0.1	-	-	-	-
	0.25	0.024	0.459	0	0.598
	0.5	0.233	0.038	-0.002	0.071
	0.75	0.539	0.001	-0.004	0.031
	0.9	0.956	0	-0.008	0.032
		Breusch-Pagan test		0.02	
T. nylanderi (So.W.)	0.1	-0.001	0.913	0.001	0.005
	0.25	0.077	0	0.001	0.008
	0.5	0.221	<0.005	0	0.672
	0.75	0.472	<0.005	-0.001	0.014
	0.9	0.806	<0.005	-0.002	<0.005
		Breusch-Pagan test		<0.005	
T. longispinosus (NY)	0.1	-0.009	0.586	0.003	<0.005
	0.25	0.197	<0.005	0.001	0.279
	0.5	0.402	<0.005	-0.001	0.144
	0.75	0.672	<0.005	-0.004	<0.005
	0.9	0.894	<0.005	-0.003	0.21
		Breusch-Pagan test		<0.005	
T. longispinosus (WV)	0.1	0.086	0.042	0.004	0
	0.25	0.363	<0.005	-0.002	0.404
	0.5	0.692	<0.005	-0.008	<0.005
	0.75	1.063	<0.005	-0.013	<0.005
	0.9	1.492	<0.005	-0.018	<0.005
		Breusch-Pagan test		<0.005	
T. nylanderi (So.S.)	0.1	0.047	<0.005	0	0.634
	0.25	0.132	<0.005	0	<0.005
	0.5	0.236	<0.005	0	<0.005
	0.75	0.368	<0.005	-0.001	0.014
	0.9	0.582	<0.005	-0.001	<0.005
		Breusch-Pagan test		<0.005	

General discussion

Throughout the last years aging in eusocial organisms attracted increasing attention following Wilson and Hölldobler's (1990) book which showed long life span in ant queens and to a lesser extent also in workers of some species. However, they also mentioned that the amount of data is insufficient to derive general conclusions from the often anecdotal information on queen and worker life span. A first comparative study emerged in 1997 demonstrating the extraordinary life spans reached by ant queens and the fact that queens of social insects live far longer than solitary insects (Keller and Genoud 1997; Keller 1998). From then on several reviews and studies focused on the proximate and ultimate factors driving life span evolution of different castes within insect societies (Parker 2011; Jemielity et al. 2005; Parker et al. 2004; Heinze and Schrempf 2008; Schrempf et al. 2005; Amdam and Page 2005; Amdam and Omholt 2002; Amdam et al. 2004; Omholt and Amdam 2004; Rueppell et al. 2005; Rueppell et al. 2007a; Rueppell et al. 2007b; Rueppell et al. 2009; Remolina and Hughes 2008; Muench et al. 2008). Most importantly, from a life-history perspective, the long life span of the queen is associated with a high fertility (Schrempf et al. 2005; Hartmann and Heinze 2003). Thus, two traits that are usually traded-off against each other (Stearns 1992) are co-occurring here. The queen is the most long-lived and most fertile organism of the colony. The opposite is true for workers which often do not reproduce but have a much shorter life span than the queen. Most intriguingly, queens and workers develop through phenotypic plasticity on the same genetic background (Evans and Wheeler 1999; Judice et al. 2004). This properties are not unique to the social insects but also apply to other eusocial species such as mole-rats where some specie also show the divergence of queen and worker life span (Dammann and Burda 2007, 2006; Dammann et al. 2011; Buffenstein 2008, 2005). A further phenomenon observed within the family Bathyergidae, which contains the social mole-rats, is the inversion of the usually observed allometric relation of body size and life span, the smallest mole-rat species (*Heterocephalus glaber*) shows the longest life span (Dammann 2006).

In this thesis I focused on the evolution of worker life span, as well as on the differences between worker and queen life span and on factors that affect the life span such as colony size. It has widely been argued, that varying extrinsic mortality leads to the evolution of different aging phenotypes in queens and workers, and that therefore the classic aging theories fully account for the observed patters (Keller and Genoud 1997; Chapuisat and Keller 2002; Heinze and Schrempf 2008). But in chapter I we showed, that if the selection is assumed to be on the colony level classic aging theories do not account for the individuals within a colony and lead to biological arguable conclusions such as “reversed trade-offs” (Parker 2011). Only Kirkwood’s disposable soma theory could be modified and applied to understand the evolution of different aging phenotypes within a colony by assigning the worker to the soma and the queen to the germ line (Kirkwood 1977). Although the level of selection is a widely debated, this approach delivers a straight forward explanation for the evolution of different aging phenotypes. The classical aging theories assume that aging evolves via a reduction in the force of selection at older ages that allow deleterious mutation to accumulate (Moorad and Promislow 2010; Medawar 1952).The hierarchical trade-off introduced in chapter I offers an approach on how the evolution of different aging phenotypes can be beneficial for the colony. Further, the approach shows that a short worker life span could actually be selected for to increase fitness on the colony level. Interestingly, this approach would lead to a stronger force of selection on the investments into the quality of workers as colony size increases, because the ergonomic benefits of a mutation leading to a reduced or increased investment would be multiplied by the number of workers in a colony. Thus, the finding of chapter III that species with larger colonies show an increasing divergence of queen and worker life span is in line with the hierarchical trade-off approach presented in chapter I.

It was proposed that the demographic properties of individuals or castes within a colony are adaptive (Schmid-Hempel 1992; Bershers and Traniello 1994; Oster and Wilson 1979; Hölldobler and Wilson 1990). Instead of being an epiphenomenon, as selection works on individuals in solitary animals, demography within social insect colonies could be directly adaptive to increase the ergonomic efficiency of the colony (Oster and Wilson 1979; Hölldobler and Wilson 1990). In the initial phases of colony development ant queens may produce smaller workers that are more efficient at brood rearing than larger workers and that are also more efficient per unit of energy investment (Porter and Tschinkel 1986). Further, the caste composition of the colony changes during colony development or after experimental caste removal (Hölldobler and Wilson 1990). In species with a monomorphic worker caste workers show a temporal task specialization (age polyethism) starting with tasks such as nursing early in life and switching later to tasks associated with higher risks such as nest repair or foraging (Camargo et al. 2007; Seeley 1982). Age polyethism is of interest to understand the evolution of worker life span and has been linked to the ergonomic optimization of colonies (Odonnell and Jeanne 1995a; Tofilski 2002; Odonnell and Jeanne 1995b; Oster and Wilson 1979). Therefore, the life span of workers may be adaptive on the colony level and directly selected for in response to the occurring tasks as well as risks associated with foraging as shown for bees and wasps (Odonnell and Jeanne 1995b; Rueppell et al. 2007a). In chapter II we showed that body size and life span of *Lasius niger* workers changed during colony development. This could again be linked to an adaptive process on the colony level that increases colony efficiency and therefore fitness. As in the mole-rat example above the life span of *L. niger* workers negatively associated with worker size. Once the colony reaches a size threshold workers switch to cooperative foraging and as a consequence the newly reared workers are shorter lived and larger than those reared at a stage where workers forage independently. As in the comparative study for chapter III queen and worker life span diverge as the size of the colony increases. It would thus be interesting to see if the same changes in worker life span occur during colony development in other social insects with monomorphic worker castes. For the honey bee the size of the colony individuals lived in showed the same effect on life span, workers live shorter in larger colonies even if they were reared under identical conditions, but the effect of the natal colony on honey bee worker life span remains to be tested (Rueppell et al. 2009). For the experiments performed for chapter II it was therefore important that the experienced colony sizes or social environment was the same for all individuals, while they were raised in differently sizes colonies, so that the effect of the natal colony on life span of the produced workers could be tested. These experiments showed that the stage of the colony which is usually defined by colony size (Mailleux et al. 2003; Cole 2009) has an explicit effect on the life-histories of workers even if the social environment is kept similar as in our experiments. One problem of the experiments performed for chapter II was that due to the pooling of the dead workers the effects of worker- and colony size on worker life span could not be fully disentangled. We decided to omit a result from chapter II because of differences in the experienced social environment (in contrast to the experimental cohorts these cohorts were housed in smaller groups and with a queen) that may have led to an interaction with the worker life span. The first nanitic workers, which are produced solely by the resources of the queen, showed an intermediate life span as compared to the two experimental cohorts, but the body size was smaller than the size of the other workers analyzed for the experiments in chapter II. Thus, body size may not be the primary factor to determine life span in *L. niger.*

Adaptive demography as a concept is closely related to the idea of the superorganism and selection on the colony level (Schmid-Hempel 1992; Wheeler 1911). With the emergence of inclusive fitness theory and kin selection (Hamilton 1964; Smith 1964), which see the unit of selection on the individual within a colony, group-level theories fell into disrepute (Bouchard and Huneman 2013). Recently, group- or multilevel selection approaches are becoming more prominent again (Wilson and Sober 1998; Okasha 2006), but both approaches are important to understand the evolution and maintenance of eusocial systems. Kin selection is more important for questions on the evolution of eusociality, whereas a group perspective is helpful for understanding colony traits within eusocial groups. In chapter I & chapter III we took the position that selection acts on the colony level. Chapter III showed that complexity increases with colony size leading to divergent life-histories of workers and queens. This finding is in line with a general rule in biological organization as shown previously (Changizi et al. 2002; Bell and Mooers 1997). Therefore, the perspective of the colony as an individual or superorganism was absolutely essential to gain this result. The same reasoning applies to chapter I where the evolution of worker life span is seen in the context of the colony as a unit of selection and is therefore adaptive. This approach delivered a straight forward explanation for the evolution of worker life span. While the proposed model remains to be tested, as the data needed was not available, both view points are essential for future research in the field of the social insects.

As introduced in chapter II, a number of factors such as body size, temperature, task, caste, nutrition and colony size affect the life-histories of workers (Parker 2011; Chapuisat and Keller 2002; Rueppell et al. 2009; Rueppell et al. 2007a; Amdam and Omholt 2002; Dussutour and Simpson 2012; Porter and Jorgensen 1981; Calabi and Porter 1989). For some factors the effect on life span may be contradictory. Body size for example may be positively or negatively associated with worker life span (Calabi and Porter 1989; Chapuisat and Keller 2002; Parker 2011). Increasing knowledge on the relationship of different factors and their influence on life span evolution in the social insects may also be beneficial to further understand the evolution of the aging process in general.

Life-history regulation is one interesting approach to understanding worker life span. It suggests that the high plasticity of life span found for honey bee workers is regulated by a number of environmental, behavioral and colony specific factors (social environment) (Rueppell et al. 2007a; Amdam and Omholt 2002; Muench et al. 2008). This approach could explain why workers especially in the honey bee, on which most of the studies on worker life span are conducted, show “increasing”, “delaying” or “reversing” senescence (Muench et al. 2008). Disentangling the regulatory and physiological mechanisms as well as understanding the regulatory anatomy that underlies the plasticity of worker is important to understand aging in the social insects and may also disclose new approaches for solitary organisms. Most importantly, the question why such a high plasticity in the aging process evolved within the social insects should be investigated closely. The model in chapter I represents first step by showing that changes in the quality of workers may be an adaptive trait whereupon selection acts directly.

After conducting the experiments for chapter II it became obvious that colony size may influence worker life span in two different ways: first the social environment and thus experienced colony size and second the colony size at which workers have been reared at modify the life span of workers. Apart from the effects on worker life span colony size is an important species specific feature of social insects that forms the basis for various life-history trade-offs (Bourke 1999; Dornhaus et al. 2012). Colony size was shown to affect division of labor and hence the number of castes and the morphological differences between castes and as shown in chapter III the life span differential between queen and worker (Changizi et al. 2002). Furthermore, larger colony size is often advantageous because of its association with increased annual survival, better fighting abilities, increased production of sexual and more complex foraging modes (Kaspari and Vargo 1995; Bourke 1999; Palmer 2004; Sorvari and Hakkarainen 2007; Beckers et al. 1989). But factors that limit colony size have gained less attention. In chapter IV we reviewed the literature on productivity in ants. The productivity of a colony can be used as a fitness measure (Jeanne and Nordheim 1996; Michener 1964; Bouwma et al. 2006). Eusocial societies represent a functional unit that is supposed to be greater than the sum of its parts. Following that it was expected that the marginal returns of a colony increase with each additional member until an optimal colony size is reached, where each additional worker adds less productivity for the colony. Per-capita productivity (colony productivity/ number of workers) was therefore expected to increase until the optimal colony size, where productivity is maximized, is reached. Michener was the first to find that per-capita productivity may already decline at size lower than the average colony size in several wasp species (Michener 1964). The review on per-capita productivity in chapter IV revealed that the so called "Michener paradox" is evident in most ant species for which data is available. The eight study species of chapter IV belong to the cavity dwelling ants, which have small average colony sizes. It was previously expected that, as a result of disturbances, these cavity dwelling species never reach sizes where per-capita productivity in maximized (Cole 1984). But our study revealed that none of study species investigated in chapter IV showed increasing per-capita productivity. We mostly found decreasing patterns of per-capita productivity which is probably associated with the individual foraging mode that leads to a depletion of resources in the surrounding of the nest site and consequently to a logistic population growth with increasing worker numbers (Cole 2009). A new approach revealed that the splitting of the colony to several nest sites (polydomy) may circumvent the decrease in per-capita productivity, because the foragers of a colony are distributed to a larger foraging area. Follow up studies on the effect of polydomy on colony productivity could show if polydomy is a general strategy to increase productivity without evolving more complex foraging modes that appear in species with larger colony sizes (Beckers et al. 1989).

As for the evolution of life span in chapters I-III, it is also important for chapter IV that a single factor (per-capita productivity) is not sufficient to explain the optimal colony size of a species. The colonies of many ant species including the ones in our study grow larger than the schedules of productivity would predict. Since larger colony size is associated with improved homeostasis and survival, per-capita productivity is not the most important factor to explain optimal colony size. Species aim to reach high levels of colony homeostasis and survival in order to be able to buffer disturbances at the cost of current productivity.

In conclusion resource allocation in social insects is an important topic. A whole colony can be seen as an (super-)organism that is build from loosely integrated and more or less specialized subunits (Hölldobler and Wilson 2009; Reeve and Hölldobler 2007; Thenius et al. 2006; Wilson and Sober 1989; Schmid-Hempel 1992). Due to the open structure and the possibility to raise colonies in the laboratory, social insects are ideal models to study processes and life-history trade-offs that are usually not measurable in a "closed" organism. Allocation strategies can thus be directly quantified as the resources flow into the colony and between individuals. For example, the investment into somatic maintenance and growth on the colony level can be measured as the investment into workers. Therefore, costs and benefits of changing life-histories of individuals within a colony can be understood and the gained knowledge can possibly be transferred to other organisms where those resource flows cannot be measured. A further project in cooperation with the Max Planck Institute for Demographic Research and the University of Regensburg aims to measure the trade-off between maintenance and reproduction within a *Cardiocondyla obscurior* colony. The goal is to understand if ant colonies trade-off maintenance against reproduction and if the quality of workers changes during colony development. In general, metabolic rates, life span and the allocation of resources can be fully measured and would help to understand the observed life-history patterns and the plasticity in worker life span.

Comparative studies on social insects are relatively rare because often the data needed are not available or are not published. It was surprising to see that data on life span in the social insects is relatively scarce, although the topic gained increasing interest in the last years. I managed to compile the largest dataset on worker and queen life span and chapter III is a result derived from this database, even though several researchers I talked to in advance where not sure if the needed data is available. Sociometric data (colony size, queen number, number of nests, worker size, alate size and number, season of reproduction, the number of matings, mode of colony reproduction, nest location, nest architecture and Queen and worker life span) is still rare and no database to store this data exists (Laskis and Tschinkel 2009; Tschinkel 1991; Keller 1998). Following that another future project could be to set up a database, so that sociometric data can be stored and accessed even if scientific journals do not publish this information.

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Authors contributions

Chapter	Idea	Work planned	Laboratory / field work	Analysis	Manuscript	Manuscript finalized
II	BHK	BHK	-	BHK	BHK	BHK,RS
II	BHK	BHK	BHK	BHK,AS	BHK	BHK,AS
III	BHK	BHK	-	BHK	BHK	BHK,RS
IV	BHK, SF, IS	BHK	SF	BHK,IS	BHK	BHK,IS,SF

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Supplemental material

Beside my work on eusociality I also worked on the life-history of the polyp hydra (*Hydra magnipapillata*). In the following publication I contributed to the analysis and the writing of the manuscript.

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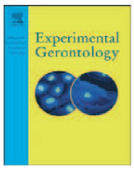

Environmental challenges improve resource utilization for asexual reproduction and maintenance in hydra

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ABSTRACT

Variation in life history can reflect genetic differences, and may be caused by environmental effects on phenotypes. Understanding how these two sources of life history variation interact to express an optimal allocation of resources in a changing environment is central to life history theory. This study addresses variation in the allocation of resources to asexual reproduction and to maintenance of *Hydra magnipapillata* in relation to differences in temperature and food availability. Hydra is a non-senescent, persistent species with primarily clonal reproduction. We recorded changes in budding rate and mean survival under starvation, which indicate changes in the allocation of resources to asexual reproduction and maintenance. In constant conditions we observed a clear trade-off between asexual reproduction and maintenance, where budding increased linearly with food intake while starvation survival stayed rather constant. In contrast, an environment with fluctuations in temperature or food availability promotes maintenance and increases the survival chances of hydra under starvation. Surprisingly, asexual reproduction also tends to be positively affected by fluctuating environmental conditions, which suggests that in this case there is no clear trade-off between asexual reproduction and maintenance in hydra. Environmental stresses have a beneficial impact on the fitness-related phenotypical traits of the basal metazoan hydra. The results indicate that, if the stress occurs in hormetic doses, variable stressful and fluctuating environments can be salutary for hydra. A closer examination of this dynamic can therefore enable us to develop a deeper understanding of the evolution of aging and longevity.

1. Introduction

Organisms differ in their growth and breeding schedules, the extent of parental investment, the number of offspring, and the investments made in body maintenance and survival. A perfect match between these life history traits of an organism can never be attained because organisms must cope with constraints, changing environments and trade-offs (Roff, 1992). Thus, the question of how resources can be efficiently allocated among growth, reproduction and survival is of particular interest.

As anticipated by life history theory, a change in one phenotypic trait may occur together with a change in another; these are referred to as trade-offs (Roff, 1992): i.e., energy that is allocated to maintenance, which in turn increases the chances of survival, cannot be used for other processes, such as reproduction (Hercus et al., 2003; Le Bourg, 2009; Boggs, 2009). The presence of trade-offs between reproduction and survival is a central feature in life history theory, and affects the evolution of longevity (Le Bourg, 2009; Stearns, 1992). Trade-offs may change in response to environmental stresses (Parsons, 2005; Rattan, 2008), and this response may differ when stresses are applied as constant or fluctuating regimes (Rattan, 2008; Parsons, 2007; Gomez et al., 2009; Marshall and Sinclair, 2010). Thermal stresses (low or high temperature) and resource scarcity induce stress responses in organisms that can lead to increased longevity, but not necessarily at the expense of their reproductive output. The improvement in the survival of an organism following exposure to mild stresses is a response known as hormesis (Parsons, 2005; Calabrese and Baldwin, 2003), and has been observed in various species across the tree of life, e.g., in yeast (Minois, 2000), *Drosophila* (Semenchenko et al., 2004; Le Bourg and Minois, 1999) and nematode worms (Yashin et al., 2001; Lee et al., 2006).

Hydra (Cnidaria, Hydrozoa) deviates from typical life histories, and offers researchers the opportunity to gain insight into the role of trade-offs in shaping age-specific life histories. Hydra reproduces mostly clonally, through budding from the body tissues by a process of mitosis and cell migration. Asexual reproduction is the main reproductive mode in hydra, while sexual reproduction occurs less frequently. Therefore, hydra is ideal for studying neglected variations in life history traits based on environmental effects. The fact that the offspring are genetically identical facilitates the observation of phenotypic variation in the allocation of resources that depend exclusively on environmental conditions.

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In hydra, three distinct stem cell lineages have been found: the epithelial stem cells (endoderm and ectoderm) provide epidermal and digestive cells, while interstitial cells provide all of the remaining cell types. The epithelial cells have been shown to have a cell cycle time of approximately three days, and to be continuously cycling (Bosch and David, 1984), so that the epithelial tissue mass of the animal doubles every three to four days (David and Campbell, 1972). Thus, the pattern of cell turnover is highly dynamic, with differentiated cells persisting for only a short time before they are lost or destroyed through apoptosis and autophagy (Chera et al., 2009). Rather than investing energy into the repair of damaged cells, hydra instead replaces whole cells, presumably because doing so is more cost-effective. These cell dynamics are of great importance for the life history of hydra, and for decisions regarding resource allocation. Furthermore, because of this dynamic balance of cell loss and cell gain, hydra is a model of continuous regeneration, and is considered to be a non-senescent invertebrate (Martinez, 1998).

Another advantage of using hydra in our experiments is that individuals kept at a constant food level attain a relatively stable maximum size (which may be species-specific). At low food levels, the size of a polyp shrinks, and the use of dead cells from apoptosis as a food source increases, as does autophagy (Bosch and David, 1984; Chera et al., 2009). With these mechanisms, the polyps can maintain their body in perpetuity, and therefore survive very low food levels or starvation for several days. Once a polyp has grown to full size (for which a constant food level is necessary), most of the newly generated cells will be channeled into budding. At this stage, resources can be allocated to asexual reproduction as well as to maintenance, and maintenance will in turn influence survival. The rate of bud production is mainly governed by temperature and food intake.

The apparent lack of senescence, the efficient asexual reproduction mechanism and the regeneration potential of hydra suggest that the response of maintenance efficiency to changing environmental conditions may be crucial to understanding the evolution of longevity and non-senescence. In many organisms, mild environmental stresses have been shown to induce a higher degree of resistance against further or new stresses, and stressed organisms have been found to have better maintenance or longer life spans than unstressed organisms. A negative effect for maintenance could be observed in overfeeding experiments conducted by Bode et al. (1977): the polyps in the experiments died, suggesting a strong decline in somatic strength. However, all of the previous experiments in which food level change and starvation were combined with an analysis of budding rate and survival lasted fewer than 30 days (Chera et al., 2009; Bode et al., 1977; Otto and Campbell, 1977). Therefore, no clear pattern with respect to resource allocation and the trade-off between maintenance and asexual reproduction could be observed.

It is the aim of our study to examine the allocation of resources to asexual reproduction and maintenance in genetically identical individuals under both controlled and stressful environmental conditions, with respect to food availability and temperature. We explore the question of whether allocation strategies are phenotypically plastic within a lifetime of a polyp, and whether they vary in response to environmental conditions. We attempt to identify the mechanism that could play a crucial role in the optimal resource allocation between survival and asexual reproduction in hydra. We compare the nutritional costs of maintenance with the nutritional costs of asexual reproduction to examine whether either one is large enough to compete for a significant fraction of the resources allocated to the other.

2. Materials and methods

2.1. Species

There are similarities in life histories across the genus *Hydra*. In this study, we use the well-studied strain *Hydra magnipapillata* 105, as this line has been kept successfully in the laboratory for over 30 years where it reproduced exclusively by clonal budding. The strain 105 does not show any signs of sexual reproduction including production of gametes. We were able to follow standard proven laboratory procedures, which includes feeding a mono-diet of *Artemia salina* nauplii (1 day post hatching). We thus benefited from the long history and experience of researchers who have worked with this model system e.g. (Martinez, 1998).

2.2. Definitions

2.2.1. Asexual reproduction = budding

We measured budding (asexual reproduction) as a reproductive rate, expressed as the average number of buds produced per hydra per unit of time. We defined asexual reproduction as a developmental process with two states based on Sanyal (1966). 1. The development of a bud starts with the tissue recruitment from the parent polyp to the bud; both are genetically identical and all three independent stem cell lineages are involved in this process (Otto and Campbell, 1977; Sanyal, 1966). 2. This process ends at the time when the bud builds its first tentacle rudiments (bud hydranth morphogenesis) and the bud separates from the parent polyp. Asexual reproduction is strongly correlated with food concentration. Furthermore, the individual size (number of cells per hydra) and the individual budding rate depend on the food intake (Otto and Campbell, 1977). Therefore, it is very important to separate budding from polyp growth, which describes the change in the size or the cell number of a polyp. We predicted that, after a long period of constant food intake, the size of the polyps would stay constant over time and within treatment groups (Otto and Campbell, 1977). Furthermore, we predicted that the size could vary among groups with different food intakes. Under constant feeding regimes with a constant number of food items per day per hydra, individuals could reach a steady state in size (number of epithelial cells), which is proportional to the food intake (Bode et al., 1977; Otto and Campbell, 1977). The respective food concentration was held constant for more than three months to allow each individual to attain this stable maximum size. We thereby ensured that 1) at the beginning of each experiment, size effects between the polyps could be nearly excluded; 2) all polyps would have acclimatized to the respective condition; and 3) newly produced buds would have nearly the same size in each treatment.

2.2.2. Food utilization

We analyzed the efficiency of energy utilization. We quantified the number of *Artemia* needed to produce one bud by calculating the number of *Artemia* per bud per time for each individual. To do this, we integrated all of the *Artemia* fed, up to the day when the last bud separated from the mother. This is an indirect measure for detecting a change in resource allocation due to environmental stresses.

2.2.3. Survival under starvation

Another way to quantify changes in the pattern of resource allocation in hydra under environmental stresses is to assess the energy needed for maintenance; i.e., survival. In hydra, the identification of a relationship between environmental stresses and survival expansion, as observed in other species, is not directly possible due to its unknown and extraordinary lifespan. Thus, the indirect measurement of life expectancy under starvation in days, as it is used in our experiments, serves as a comparable approach. Significant differences in such an artificial mortality curve can then be explained as differences in the allocation of resources to maintenance. To avoid effects of cell number or size of polyps on this parameter (because survival can be positively correlated with the number of cells per polyp, as mentioned above) we started with an experiment in which we fed groups of polyps at different constant food levels (between 0.2

and 20 *Artemia* per day and polyp). The feeding period was followed by a final starvation time to test the influence of the food level on the survival under starvation (= maintenance). We predicted that, because the size of a polyp depends on food level, starvation survival would be higher in polyps fed with more *Artemia* prior to starvation. This is because these polyps contain more cells and thus more substance (Otto and Campbell, 1977) undergoing apoptosis and autophagy (Bosch and David, 1984; Chera et al., 2009), which should result in a longer life span without food.

2.3. Experimental design

2.3.1. Culture conditions

We cultured individuals in plastic multi-well culture plates with a medium containing 0.05 mM $NaHCO_3$, 1 mM $CaCl_2$, 0.1 mM $MgCl_2$, 0.001 mM $MgSO_4$, 0.003 mM KNO_3 in deionised water, and maintained them in incubators at 18 °C with a 12 h-light, 12 h-dark regime (= control environment). For the experiments, each polyp was kept separately in a single container containing 8 ml of hydra medium on plastic 6-well plates. Hydra polyps were fed with freshly hatched nauplii of *Artemia salina*. Exact numbers of these *Artemia* can be fed directly to the hydra, offering a simple and practical way of manipulating the resources given to individuals. In all of the experiments, all of the polyps were checked for complete food intake after feeding. We are aware that the exclusive use of *Artemia* is not natural (mono-diet) as compared with multiple food sources in the wild. But using *Artemia* as food source offers the opportunity to compare the levels of food intake between individuals since the *Artemia nauplii* we used were nearly constant in size and energy level (1 day post hatching).

To reduce age effects within/between the experimental groups, we used polyps of a similar age in all experiments. We collected newly released buds from an existing adult polyp culture in our lab within 3–5 months. All individuals used for the experiments in this study were taken out of this pool.

2.3.2. Experiments

The exact details of the individual experiments and their simulated environmental stresses (like feeding regime, hunger periods, temperature) are depicted in Table 1. Treatments with lower food levels lasted longer to ensure that the polyps did not die due to the low food level itself. The results of the following experiments, in which we treated groups of hydra with varying temperatures and starvation regimes, are compared and discussed with respect to the possible size effect on life expectancy under starvation.

(1) Constant food levels
We studied the effect of different food levels on asexual reproduction and survival under laboratory conditions (18 °C and 12 h dark: 12 h light conditions). Food level responses are measured as the reproductive rate, food utilization per bud and the survival time (mean life expectancy under final starvation). Groups of hydra with constant but differing mean food levels between 0.2 and 20 *Artemia* per day per hydra were compared (Table 1).

(2) Variation in mean food level
Here we compared both the budding rate and the life expectancy under starvation in two scenarios: low food (1.3 *Artemia* per day per hydra) after 119 days of high food (3 *Artemia* per day per hydra), and high food after 119 days of low food. Both of these outcomes are compared to the outcomes of control groups (Table 1).

(3) Food scarcity in-between
Two additional experiments were run to analyze the effect of a limited starvation time: the low-food group was fed with 1.3 and the high-food group with 3 *Artemia* per day per hydra. We ceased feeding after 101 days in both groups for 61 days. Thereafter, the original feeding schedule was resumed for an additional 61 days (Table 1), after which life expectancy under final starvation was measured.

(4) Temperature and food concentration
To test the influences of temperature on hydra longevity, asexual reproduction and food utilization, we conducted two different experiments. In the first experiment, hydra were kept under a constant temperature regime (10 °C or 18 °C) and were fed either 1 or 4 *Artemia* per day per hydra (Table 1).

In the second experiment, we exposed hydra polyps to a temperature cycle (18–14–10–6–10–14-18 °C; each temperature for four weeks). One group was fed 1.3 *Artemia* per day per hydra, while a second group was fed 3 *Artemia* per day per hydra. (Table 1).

2.3.3. Statistics

SPSS software was used for the statistical analysis of the obtained data. We tested the hypothesis that mild stresses (temperature and food variation or food scarcity) would have an effect on lifetime asexual reproduction, food utilization and starvation survival, when compared to constant conditions without stresses using an analysis of variance (one-way ANOVA). The analysis of variance was followed by Tukey *post-hoc* comparisons to test for differences between the treatments with and without mild stresses. To test the effects of different environmental conditions and their interactions on the survival and reproduction of hydra, we compromised on the number of individuals in each treatment and thus reduced the statistical strength of the analysis for feasibility reasons.

3. Results

3.1. Constant food

Fig. 1 shows how asexual reproduction and survival were affected by the overall mean daily food intake. Increasing resource levels caused a linear increase in budding rates (linear model: $F_{1,287} = 3317$; $p<0.001$; $r^2 = 0.92$ and ANOVA, $F_{10,287} = 383$, $p<0.001$). At the food level of 0.21 *Artemia* per day, no buds developed (Fig. 1). The food utilization per bud per hydra was nearly constant across all food levels (with the exception of food treatment<1 *Artemia* per day), with an average requirement of 16 *Artemia* per bud ($F_{8,233} = 1.9$; $p = 0.069$; data not shown).

With increasing food intake, starvation survival was at a fairly constant level across all food treatments, with more than 0.6 *Artemia* per day (linear model: $F_{1,256} = 3.1$; $p = 0.075$; $r^2 = 0.012$), but there were significant differences between single groups (Fig. 1; ANOVA, $F_{10,287} = 47$, $p<0.001$). At higher food levels (<3 *Artemia*), the mean starvation survival was nearly 80 days. At 0.21 *Artemia* per day per hydra, the mean starvation survival of 48 days was significantly lower (Fig. 1; Tukey *post-hoc*: $p<0.05$). The highest values of starvation survival could be observed at one and 1.3 *Artemia* per day per hydra treatments (Fig. 1). Surprisingly, these levels showed a higher starvation survival than groups with higher food intake. The nearly constant level of life expectancy under starvation over all higher food concentrations showed that, in our experiments, starvation survival (= maintenance) seemed to be nearly independent of food intake (except the minimum food levels). It thus appears that starvation survival behaves independently of size and the number of cells per polyp, respectively. At a very low food level (<1 *Artemia* per day per polyp), we cannot exclude an effect of size or cell number.

Table 1
Information about experiments.

	Number of Individuals	First feeding regime				Changing treatments	Second feeding regime		
		Temperature °C	*Artemia* per day per Hydra	Feeding frequency: *Artemia* per day	Feeding days		*Artemia* per day per Hydra	Feeding frequency: *Artemia* per day	Feeding days
Constant food	30	18	0.21	1–0–0–0–0– 1–0–0–0–0	112	– –	–	– –	–
	24	18	0.57	2–0–0–0– 2–0–0	112	– –	–	– –	–
	24	18	0.79	3–0–0–2–0– 0–2–0–0	95	– –	–	– –	–
	30	18	1.00	2–0–2–0– 3–0–0	95	– –	–	– –	–
	24	18	1.29	3–0–3–0– 3–0–0	95	– –	–	– –	–
	24	18	2.43	3–2–3–2– 3–2–2	95	– –	–	– –	–
	24	18	3.00	3–3–3–3– 3–3–3	95				
	30	18	4.00	4–4–4–4– 4–4–4	77	– –	–	– –	–
	24	18	6.00	6–6–6–6– 6–6–6	77	– –	–	– –	–
	24	18	10.00	10–10–10–10– 10–10–10	77	– –	–	– –	–
	24	18	15.00	15–15–15–15– 15–15–15	77	– –	–	– –	–
	24	18	20.00	20–20–20–20– 20–20–20	77	– –	–	– –	–
Different temperatures	30	10	1.00	1–1–1–1– 1–1–1	65	– –	–	– –	–
	30	10	4.00	4–4–4–4– 4–4–4	65	– –	–	– –	–
Changing food level	24	18	1.29	3–0–3–0– 3–0–0	119	– –	3.00	3–3–3–3– 3–3–3	91
	24	18	3.00	3–3–3–3– 3–3–3	119	– –	1.29	3–0–3– 0–3–0–0–	91
Scarcity in–between	24	18	3.00	3–3–3–3– 3–3–3	102	Scarcity of 61 days	3.00	3–3–3– 3–3–3–3	63
	24	18	1.29	3–0–3–0– 3–0–0	102		1.29	3–0–3– 0–3–0–0–	63
Temperature-gradient	24	18–14–10– 6–10–14–18	1.29	3–0–3–0– 3–0–0	252	Weeks per temperature: 8–4–4–4– 4–4–8	–	– –	–
	24		3.00	3–3–3–3– 3–3–3	252		–	– –	–

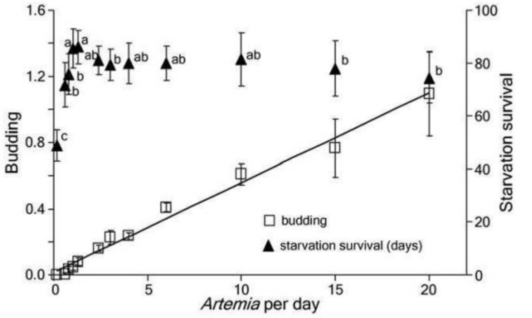

Fig. 1. Relationship between daily budding rate and remaining life expectancy under starvation after various food regimes. Different lower case letters indicate significant differences in life expectancy (one-way ANOVA, Tukey *post-hoc* test, $p<0.05$).

3.2. Variation in mean food level

To simulate the varying levels of food abundance in the natural environment, we performed two different experiments: by changing food levels from high to low, and from low to high. We then compared these groups to a control group in which food levels remained constant. In general, the reproductive effort was affected by the direction of the changing food concentration. The mean budding rate in the 1.3 *Artemia* per day per hydra treatment was significantly higher in the high-low group than in the low-high food and in the control group (Fig. 2A; ANOVA, $F_{2,71}=130$, $p<0.001$; Tukey *post-hoc*: $p<0.05$).

The efficiency of food utilization was positively affected by the direction of the changing food concentration. When the food intake was reduced from 3 to 1.3, hydra individuals used a lower number of *Artemia* to produce a bud (Fig. 2B; ANOVA, $F_{2,71}=47$, $p<0.001$). Under constant food conditions, hydra needed an average of 13 *Artemia* per bud, but after food levels were lowered, this fell to nearly 10 *Artemia* per bud (Tukey *post-hoc*: $p<0.05$). At low food levels, the budding rate was reduced as well, while food utilization increased, such that relatively more buds were produced per amount of food.

In addition, the starvation survival was significantly affected by the direction of the changing food concentration (ANOVA, $F_{3,95}=70$, $p<0.001$). A strong increase in mean starvation survival (91 ± 9 and 101 ± 8 days) for changing food treatments from 1.3 to 3 and 3 to 1.3 *Artemia* per day (Fig. 3A) was observed when compared to the constant food concentration at 1.3 (86 ± 6 days) and 3 *Artemia* per day per hydra (79 ± 6 days) (Tukey *post-hoc*: $p<0.05$). Again, the individuals in the group changing from higher to lower food intake show longer starvation survival than the individuals in the group with higher food intake as the final food level (3 *Artemia* per day). In the constant controls, the polyps fed 1.3 *Artemia* per day per hydra were living longer under starvation than their control counterparts, which were fed 3 *Artemia* per day (Fig. 1).

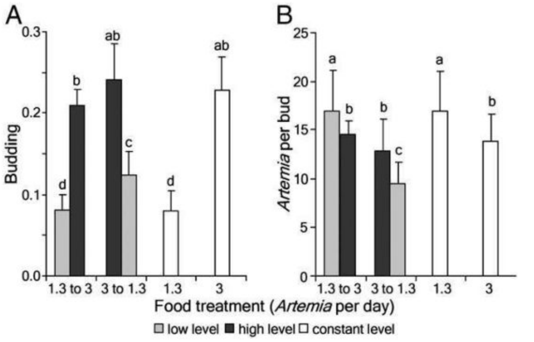

Fig. 2. Relationship of budding rate (A) and food utilization (*Artemia* per bud) (B) of one treatment with change mean in food concentration from 1.3 to 3 *Artemia* per day and the second treatment with change in mean food concentration from 3 to 1.3 *Artemia* per day as well as two control treatments with constant food levels (1.3 and 3 *Artemia* per day). Different lower case letters indicate significant differences in budding rate or in food utilization between the various food treatments (one-way ANOVA, Tukey *post-hoc* test, $p<0.05$).

3.3. Food scarcity in-between

To further test the influence of hunger time and food level on life history traits, we conducted a series of experiments with a longer hunger period between two feeding regimes with a constant food level. The groups were subject to a high and a low food regime, with 3 and 1.3 *Artemia* fed per day per hydra, respectively. Both groups were exposed to a fasting period of 61 days before re-feeding. In the 1.3 *Artemia* treatment, no change in asexual reproduction between before and after the fasting time and the control group could be observed (Fig. 4A; ANOVA, $F_{2,71}=2.4$, $p=0.098$). In the 3 *Artemia* treatments, the reproductive success after the fasting time was significantly lower (Fig. 4A; ANOVA, $F_{2,71}=4.1$, $p=0.028$). The food utilization was not affected by the starvation period (data not shown; ANOVA, $p>0.13$ for 1.3 *Artemia* treatment; $p>0.43$ for 3 *Artemia* treatment).

The long fasting period strongly affected the starvation survival in both treatment groups (Fig. 3B). Compared to the control group (Fig. 1 and Fig. 3B), without a hunger period, the mean starvation survival increased by 29 days and 17 days for the 3 and 1.3 *Artemia* treatments, respectively and were significantly higher (ANOVA, $F_{3,95}=136$, $p<0.001$). But again, we did not detect higher rates of survival under starvation for individuals with higher food intake (3 *Artemia*) than for those with lower food intake (1.3 *Artemia*) in the treatment groups. Instead, the trend was found to be opposite in the controls.

3.4. Temperature

3.4.1. Constant temperature

To test the consequences of a constant low temperature on life expectancy and asexual reproduction, we compared two food treatments with one and four *Artemia* per day per hydra at two different temperatures: 10 °C and 18 °C.

The results of these experiments suggest that temperature has a strong influence on starvation survival (Fig. 3C) and asexual reproduction (Fig. 4B). The asexual reproduction at 10 °C decreased dramatically relative to the 18 °C treatments in both food treatments (Fig. 4B; ANOVA, $F_{3,119}=498$, $p<0.001$). Moreover, low temperature increased mean starvation survival significantly in both food treatments (Fig. 5; ANOVA, $F_{3,119}=172$, $p<0.001$), by 57 days for the four *Artemia* treatments, and by 46 days for the one *Artemia* per day per hydra treatments (Fig. 3C). Within a temperature treatment, mean starvation survival was independent of food concentration (Fig. 3C; Tukey *post-hoc*: $p>0.05$).

3.4.2. Temperature cycle

In this experiment, the effects of mild temperature stress, simulating winter conditions, were tested: survival after starvation (Fig. 3D) was measured following the temperature cycle, and the budding rate (Fig. 5A) was measured and compared at the beginning and at the end of the temperature cycle at 18 °C. In both food treatments (3 and 1.3 *Artemia* per day and per hydra), the budding rate increased significantly relative to the results at 18 °C before (first 30 days) and after (last 30 days) the temperature cycle, and also relative to the control groups at constant 18 °C (Fig. 1; ANOVA, $F_{2,71}=11$, $p<0.001$ for 1.3 *Artemia* treatment; $F_{2,71}=88$, $p<0.001$ for 3 *Artemia* treatment).

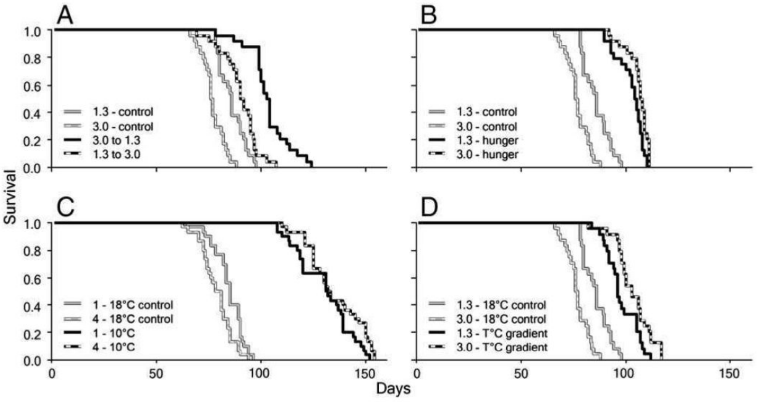

Fig. 3. Survival curves starting at the onset of starvation for different treatments: A, survival curves for change in mean food concentration from 3 to 1.3 *Artemia* per day and from 1.3 to 3 *Artemia* per day compared to the control group (constant 1.3 and 3 *Artemia* per day); B, survival curves for treatments with intermediate hunger period of 61 days and 1.3 and 3 *Artemia* per day feeding regimes. Control group without hunger but same feeding regime; C, survival curves for two different food regimes (4 and 1 *Artemia* per day) and two temperature treatments (10 and 18 °C); D, survival curves for the temperature gradient treatment (1.3 and 3 *Artemia* per day) compared to the control group (constant temperature; 1.3 and 3 *Artemia* per day).

The efficiency of food utilization measured as *Artemia* per bud per hydra improved significantly after the temperature cycle in both food treatment groups, compared to the control group with a constant temperature of 18 °C (Fig. 5B; ANOVA, $F_{2,71} = 17$, $p < 0.001$ for 1.3 *Artemia* treatment; $F_{2,71} = 38$, $p < 0.001$ for 3 *Artemia* treatment). The changes in food utilization after the temperature cycle were independent of the food concentration (Fig. 5B; Tukey *post-hoc*: $p > 0.05$).

The individual survival after final starvation was significantly affected by the temperature cycle (Fig. 3D; ANOVA, $F_{3,95} = 77$, $p < 0.001$). The mean starvation survival of these individuals was 29 (3.0 *Artemia*) and 20 (1.3 *Artemia*) days longer than the mean survival starvation of individuals under a constant temperature of 18 °C. Interestingly, the individuals that underwent the 3 *Artemia* treatment had a significantly longer mean starvation survival (105 ± 4 days) than those that underwent the 1.3 *Artemia* treatments (99 ± 7 days) following the temperature cycle (Tukey *post-hoc*: $p < 0.05$). In contrast, under a constant temperature of 18 °C, the differences between food treatments were reversed (Tukey *post-hoc*: $p < 0.05$).

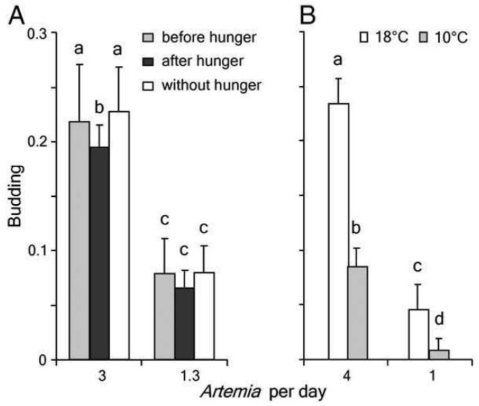

Fig. 4. The budding behavior of two different treatments: A, mean budding rate for 1.3 and 3 *Artemia* per day with intermediate hunger period of 61 days in both treatments. Control groups without hunger (1.3 and 3 *Artemia* per day); B, mean budding rate under different but constant food regimes (4 and 1 *Artemia* per day) and constant temperature (10 and 18 °C). Different lower case letters indicate significant treatment effect (one-way ANOVA, Tukey *post-hoc* test, p<0.05).

4. Discussion

4.1. Environment with constant food and temperature levels

A polyp's size, the size of its buds and its budding rate depends on food intake (Otto and Campbell, 1977). Once a polyp has grown to full size, most of the newly generated cells will be channeled into budding. At this stage, resource allocation to growth is negligible. Resources can be allocated to budding as well as to maintenance, which in turn influences survival.

At constant temperatures and at constant food services, budding rates showed a linear increase with increasing food levels (Fig. 1), thus confirming previous studies (Otto and Campbell, 1977). In contrast, starvation survival was not positively affected by different constant food levels. Survival thus appears to increase with food intake only up to a critical level (one *Artemia* per day). Once this critical food level is reached, the variation in starvation survival rates remains very small, with no trend towards an increase in survival for higher feeding regimes. We anticipated that higher food levels would

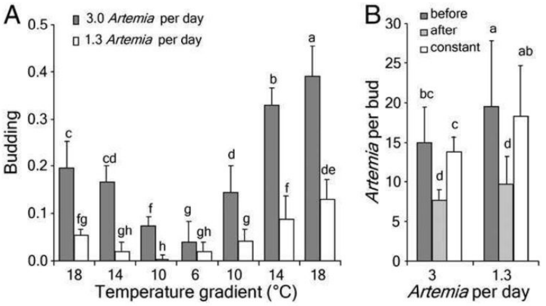

Fig. 5. Budding rate (A) of individuals exposed to different temperatures in a temperature cycle for 1.3 and 3 Artemia per day and energy utilization (*Artemia* per bud; B) at 18 °C before and after the temperature cycles compared with the control treatment at constant 18 °C.

increase the cell number per polyp (Otto and Campbell, 1977), and would therefore increase survival during starvation. Instead, we observed that larger polyps did not increase their starvation survival. Moreover, it seems that being kept at very high food levels for a long time may even be disadvantageous for hydra's survival probability under starvation.

It appears that larger polyp size comes at a cost, which indicates that a trade-off exists between growth, asexual reproduction and survival. More food increases the budding rate linearly, and enough food (>3 *Artemia*) increases the cell number per polyp, which results in a lower surface area to volume ratio than for polyps with a lower number of cells. But it seems that larger polyps lack an advantage by having a lower surface area to volume ratio with respect to energy-use efficiency, as predicted by the Bergmann's rule, at least with measured survival during starvation. By contrast, the similar survival for survival for polyps under starvation that were previously exposed to lower food levels indicates that a shift occurs in resource allocation to growth and budding, while maintenance allocation is kept at a constant or even lower level, which is indicated by the reduced starvation survival among previously highly fed groups. Consequently, smaller and less fed polyps seem to have a more balanced resource allocation, and they proportionally invest more energy in maintenance than the highly fed polyps.

Another explanation for the observed survival pattern could be that constant excessive food supply leads to more inefficient energy consumption, affecting basically all physiological functions in hydra. Such a wasteful handling of energy could lead to a lowered allocation to maintenance or a proportional increase prior to starvation, thus resulting in a similar or slightly shorter survival during starvation compared to less fed hydra. After a sudden cessation of food supply, the lower energy efficiency results in a quicker decline in cell numbers per polyp, because proportionally more cells are used as food and energy in autophagy and apoptosis to cover the cost of starvation.

A third explanation would be that, at high food levels, a decline in the somatic strength (i.e., maintenance efficiency) of the polyp results in increasing costs per polyp for maintenance, and leads to a lower life expectancy under starvation. This decline in polyp health could be explained by increasing maintenance costs due to the need among larger polyps to allocate more energy to repairing and building defense mechanisms to avoid the accumulation of permanent deleterious damages in cells. These higher costs are a consequence of the persistence of high metabolic rates per cell that were established during times when enough or too much food was available. Similar effects were observed by Bode et al. (1977) and Galliot and Ghila (2010), as their overfeeding experiments resulted in the natural death of the polyps.

Constant lower temperature treatments at 10 °C force also a trade-off in both life history traits: maintenance and asexual reproduction. The remarkable increase in starvation survival and the decreased budding rate at a constant temperature of 10 °C, compared to 18 °C, demonstrates that, at low temperature, the allocation to asexual reproduction is lowered, while the allocation to maintenance increases. These results are similar to those seen previously in hydra (Schroeder and Callaghan, 1981; Schroeder and Callaghan, 1982; Park and Ortmeyer, 1972). At low temperature, a reduced asexual reproduction could be observed, while polyp size was sometimes found to increase. The finding of an increase in life span may not be too surprising considering the reduction in the metabolic rate and the potential for a greater investment in body size under constant lowered temperature (McCabe and Partridge, 1997; Bochdanovits and De Jong, 2003; Castilho et al., 2007). Another possible explanation could be that there is no change in the allocation resources; instead, the observed patterns could be simply the result of the reduction in the total metabolic rate, which lowers the budding and cell division rates, and thereby promotes longer starvation survival under low temperatures.

4.2. Environment with variable food and temperature levels

In these experiments, we tested the abilities of hydra to withstand variable environments. After hunger periods between two constant feeding times, starvation under final starvation was largely extended (Fig. 2B). Imposing a short period of food deprivation is one of the tools for manipulating the maintenance investments of various organisms that has been most frequently investigated in studies on aging (Hulbert et al., 2007), and the effects of this technique have been observed in *C. elegans* (Lee et al., 2006; Lakowski and Hekimi, 1998), in some fruit fly species (Partridge et al., 2005; Carey et al., 2008), rodents (Masoro, 1988) and rotifers (Verdonesmith and Enesco, 1982; Ozdemir, 2009).

Not only the absolute abundance of food, but also the direction of change in food abundance is important for hydra. In our study, a change from an abundant food supply to a scarce or moderate food supply was found to result in higher asexual reproduction compared to the moderate food supply of the opposite direction treatment. Secondly, we observed an increase in the energy utilization ratio relative to a constant food supply (i.e., the number of *Artemia* per bud) (Fig. 2B). In the high to low food treatment, food utilization—i.e., food conversion efficiency—increased in hydra. This may seem surprising, but can be explained by the fact that food limitation is mostly associated with harsher conditions, and, consequently, with high extrinsic mortality in the field (predation and eutrophication). A relatively high budding rate allows the population to survive such conditions for a while. Consequently, there should be consistently strong directional selection to enhance food utilization whenever food is scarce. In contrast, increasing food supply leads to reduced food utilization. Starvation survival increased in both food treatments, irrespective of the direction of change and of asexual reproduction, though the survival increase was even more pronounced in the high to low feeding treatments (Fig. 3A). The increase in life expectancy indicates an increase in the maintenance performance and an increase in the maintenance efficiency of the polyps as a result of the changing food regimes prior to starvation. This interesting response could be related to a hormetic effect, i.e., a beneficial effect on the starvation survival or on the polyp maintenance of hydra under hormetic doses of increased environmental stress (Calabrese and Baldwin, 2003).

Most interestingly, fluctuation in temperature was found to have a beneficial effect on both asexual reproduction and maintenance in hydra. In a temperature cycle that simulates the seasonal variability in temperature in the field at low extrinsic mortality and high food abundance, hydra polyps were found to survive at a temperature of 6 °C. At low temperatures, asexual reproduction was shown to decline, but after the temperature was raised again to 18 °C, asexual reproduction was found to increase substantially at each food level compared to the same levels before reaching the lowest temperature (Fig. 5A). After temperature cycles were imposed, starvation survival was found to increase significantly compared to polyps in constant 18 °C regime (Fig. 3D). In this case, the short period of lower temperature was shown to induce an increased allocation of resources into both asexual reproduction and maintenance after the temperatures returned to higher levels. We suggest that the resources that were allocated to maintenance during lowered temperatures were reinvested into an efficient asexual reproduction and maintenance. These positive processes were sustained for several weeks at a constant 18 °C, even during starvation. Consequently, habitats with lower temperatures appear to support strategies in which individuals allocate more resources to asexual reproduction and an individual's maintenance following these lower temperatures, thereby increasing survival during starvation as well.

4.3. Causes and consequences

We had anticipated that *Hydra* would have evolved mechanisms by which the energy requirements for reproduction and survival can be met to optimize fitness in a given environment, such that energy saved by a reduction in asexual reproduction can be used to increase maintenance, i.e., survivorship. The results of our study provide direct evidence that both asexual reproduction and maintenance in particular benefit from environmental stresses.

A very interesting observation in our study is that polyps in a steady-state condition (concerning all morphogenetic processes like homeostasis in cell production and loss, animal size and budding), have a lower starvation survival than polyps in more stressful conditions, in which homeostasis was interrupted. A higher resistance to stress, which leads to increases in maintenance and reproduction efficiency, could be attributed to the beneficial effects that may result from exposure to low doses of mild stress, a response known as hormesis (Rattan, 2008; Calabrese and Baldwin, 2003; Mangel, 2008). But the mechanisms of such benefits are multi-faceted and difficult to assess. In general, hormesis induces stimulation of protective cell mechanisms, leading to an improvement in overall cellular functions and performance (Rattan, 2008). Stress triggers a signal transduction network, which can result in an enhancement of metabolic efficiency, thus allowing the body to maintain, survive and reproduce with a lower supply of energy. These processes, which can play a decisive role in the development of higher resistance to environmental stress—like cell proliferation (with constant cell cycling lengths), autophagy and apoptosis—are responsible for the morphogenesis and the survival of the polyps in general (Galliot and Ghila, 2010). Constant conditions with sufficient food and low extrinsic mortality hazard lead to homeostasis or steady-state conditions in organisms: when cell turnover, cell production and cell loss, as well as the polyp size (cell number) and asexual reproduction, are stable, levels of autophagy and phagocytosis of apoptotic cell bodies are low or negligible. For polyps that have acclimated to constant environmental conditions over a period of several months, a sudden change in environmental conditions creates a dramatic break in their homeostasis. Such breaks can influence the morphogenetic processes, resulting in rapid activation of apoptosis and autophagy, and in a decrease in the epithelial cell cycle length (Bosch and David, 1984; Bode et al., 1977). Thus, stress conditions can favor the metabolic efficiency and “wake up the polyps from their dormancy”; the cellular and developmental reactions of the polyp to stress enhance simultaneously metabolic and utilization rates of resources. This adjustment response to new environmental conditions should lead to an improvement in metabolic and physiological functions, and result in both greater maintenance efficiency and a higher survival under starvation conditions. Given that hydra has a plastic resource allocation system and can increase its maintenance efficiency through environmental stresses, the question of whether this also produces long-term benefits arises, as hormetic effects were also reported to be potentially transient effects (Stebbing, 1982). To answer this question, it is necessary to know the memory retention time of previous specific stressors for the physiological processes in a single hydra polyp or a population. Although we did not analyze the memory retention time of previous stressors to hydra in this study, there are two facts that lend support to the possibility that the patterns we observed are transient effects (Stebbing, 1982). First, the dynamic equilibrium of cell gain and cell loss leads to a continuous renewing of all cells in a polyp body within a few weeks (Campbell, 1967). Consequently, there are supposed to be no long-lived cells in the hydra body, making it difficult for the organism to maintain a long-term memory (Terman and Brunk, 2005). Second, hydra is exposed to dramatic environmental changes in its natural environment, where temperature and the availability of resources (in addition to other factors) vary greatly in time and space. Hence, we suggest that a long-term memory retention time may not be necessary, as fluctuations in the environment occur frequently and allow for recurrent and continuous improvement of somatic maintenance efficiency via hormetic-dose stress responses. Stress itself does not need to induce a lasting adaptive and heritable response in hydra.

Our results are obtained from a laboratory strain of hydra. Our finding of improved reproduction and maintenance after mild stresses found under laboratory conditions cannot directly be linked to responses in the wild. Because, in the wild we can assume that irregular and unexpected multiple changes of environmental conditions occur, which can lead to interaction effects of environmental conditions. Additional experiments in consideration of the possible interaction effects of the natural environmental have to be done in future.

5. Conclusion

Hydra presents an interesting deviation from typical life-histories: It appears to show no senescence, providing an outstanding opportunity to gain insights into the role that trade-offs may play in shaping age-specific life-histories. Therefore, a set of laboratory experiments investigated how trade-offs between reproduction and maintenance (here measured as survival under starvation) are modified under environmental stresses. For our isogenic hydra strain, lacking both genetic variation and generalist traits, we observed a clear trade-off between asexual reproduction and maintenance under constant conditions with maintenance staying rather constant while budding increased linearly with food intake. Moreover, under fluctuating environmental conditions both reproduction and maintenance can benefit from these stresses, i.e. producing a hormetic stress response. Under recurrent hormetic stresses polyps may emerge stronger in terms of health and reproductive activity with time. Such recurrent environmental stresses may have contributed in the evolution of the extraordinary aging pattern without senescence in hydra including its hormetic stress response ability. Hydra's constant cell turnover and the resulting regenerative capacity may play a crucial role in its remarkable plasticity of resource allocation responses including its hormetic abilities.

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