

The influence of individual and colony level  
variation in behavior on colony performance in  
ants

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*“The most general organismal character of the ant-colony is its individuality. Like the cell or the person, it behaves as a unitary whole, maintaining its identity in space, resisting dissolution and, as a general rule, any fusion with other colonies of the same or alien species. This resistance is very strongly manifested in the fierce defensive and offensive cooperation of the colonial personnel. Moreover, every ant-colony has its own peculiar idiosyncrasies of composition and behavior.”*

William Morton Wheeler (1911)

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## **Abstract**

In my dissertation I investigated the influence of behavioral variation between and within ant colonies on group performance. In particular, I analyzed how evolution shapes behavior in response to ecological conditions, and whether within-group diversity improves productivity as suggested by theory. Our field and laboratory experiments showed that behavioral diverse groups are more productive. Different aggression levels within colonies were beneficial under competitive field situations, whereas diversity in brood care and exploratory behavior were favored in non-competitive laboratory situations. We then examined whether population density and social parasite presence shape aggression through phenotypic plasticity and/or natural selection. The importance of selection was indicated by the absence of density or parasite effects on aggression in a field manipulation. Indeed, more aggressive colonies fared better under high density and during parasite attack. When analyzing the proximate causes of individual behavioral variation, ovarian development was shown to be linked to division of labor and aggressiveness. Finally, our studies show that differences in the collective behavior can be linked to immune defense and productivity. My dissertation demonstrates that behavioral variation should be studied on multiple scales and when possible combined with physiological analyses to better understand the evolution of animal personalities in social groups.

## **Zusammenfassung**

In meiner Dissertation untersuchte ich den Einfluss von Verhaltensvariabilität zwischen und innerhalb von Ameisenkolonien auf ihre Produktivität. Hierbei analysierte ich wie sich im Laufe der Evolution das Verhalten an ökologische Bedingungen anpasst, und ob Diversität im Verhalten innerhalb einer Kolonie die Produktivität erhöht, wie es theoretische Modelle vorhersagen. Unsere Freiland- und Laborexperimente zeigten, dass Kolonien mit höherer Diversität tatsächlich produktiver waren. Kolonien mit unterschiedlich aggressiven Tieren hatten einen Vorteil in einer kompetitiven Freilandsituation, während Variabilität im Brutfürsorge- und Explorationsverhalten unter nicht-kompetitiven Laborbedingungen förderlich waren. Als Nächstes untersuchten wir, ob die Populationsdichte oder die Präsenz von Sozialparasiten durch phänotypische Plastizität und/oder natürliche Selektion einen Einfluss auf die Aggression hat. Selektion stellte sich als wichtiger heraus, da sich die Kolonien durch Manipulation von Dichte und Sozialparasiten im Freiland nicht in ihrer Aggression verändern ließen. In der Tat kamen aggressive Kolonien besser mit hoher Dichte und der Invasion von Parasiten zurecht. Die Analyse der proximativen Ursachen individueller Verhaltensunterschiede ergab, dass die Ovarienentwicklung mit der Arbeitsteilung und der Aggression zusammenhängt. Schließlich zeigen unsere Studien auf Kolonieniveau, dass auch Unterschiede in kollektiven Verhaltensweisen zwischen Kolonien mit der Produktivität und interessanterweise auch mit der Immunabwehr zusammenhängen. Demzufolge sollte für ein tieferes Verständnis für die Evolution von Persönlichkeitsmerkmalen bei Tieren in sozialen Gruppen, Verhaltensvariation auf mehreren Ebenen untersucht, und wenn möglich mit physiologischen Analysen kombiniert werden.

## Introduction

*“It may metaphorically be said that natural selection is daily and hourly scrutinizing, throughout the world, the slightest variations; rejecting those that are bad, preserving and adding up all that are good; silently and insensibly working, whenever and wherever opportunity offers, at the improvement of each organic being in relation to its organic and inorganic conditions of life. We see nothing of these slow changes in progress, until the hand of time has marked the lapse of ages.”*

Charles Darwin (1859)

Charles Darwin was the first to recognize that differential reproductive success due to variation in inheritable traits invariably leads to natural selection and thus to evolution. Several years after he published *On the Origin of Species*, Spencer (1866) coined the expression “survival of the fittest” to describe natural selection. But what exactly is “fitness”? Hunt and Hodgson (2010) pointed out that finding an operational definition of fitness has been problematic, because it is difficult to find a common measure for different species that reliably predicts fitness in all biological contexts. Most scientists agree that fitness measures the success of contributing descendants to the next generations. Hence, Hunt and Hodgson (2010) defined fitness as “a measurable feature of alleles, genotypes or traits of individuals that predicts their numerical representation in future generations”. In many organisms, morphological traits such as body size or shape are known to have a genetic basis and are often correlated with fitness (Mousseau and Roff 1987). Among the most famous examples of natural selection influencing size and form are Darwin’s finches. Studies by Rosemary and Peter Grant revealed that environmental heterogeneity was associated with morphological variation, and that body size, bill size and bill shape not only impact foraging efficiency, but more importantly survival (Grant et al. 1976; Boag and Grant 1981).

### **Animal personality**

Individual differences in behavior, however, were traditionally regarded as “non-adaptive variation that surrounds an adaptive mean” (Wilson 1998). While psychologists like Yerkes (1939) had already studied animal personalities in the first half of the last century, most behavioral ecologists avoided to use the term personalities for animals, because it was seen as



an anthropomorphic unscientific expression (Sih et al. 2010). They have only recently recognized that consistent individual differences in animal behavior can be seen as a personality (Gosling 2001), temperament (Réale et al. 2007) and behavioral syndrome (Sih et al. 2004). From an evolutionary and ecological point of view, animal personalities have become a highly attractive study area for several reasons. First, scientists have been able to show that consistent individual differences across time and/or contexts are partly heritable (e.g. exploratory behavior in great tits; Dingemanse et al. 2002). Second, personalities have been shown to influence fitness in numerous species (meta-analysis by Smith and Blumstein 2008). For example, aggressive Ural owl mothers are able to rear more offspring (Kontiainen et al. 2009) and bolder bighorn ewes have higher survival under cougar predation (Réale and Festa-Bianchet 2003). Third, suites of correlated behavioral traits can help to explain behavioral carryovers. Behaviors that seem non-adaptive in one context might be beneficial in another. A well-known example is aggressive behavior in the fishing spider, *Dolomedes triton* (Johnson and Sih 2005): high aggressiveness in the mating context is costly, because it leads to precopulatory sexual cannibalism. Despite these costs, precopulatory cannibalism is not removed by natural selection, because aggressiveness is beneficial in the context of foraging: highly aggressive individuals are voracious, and have a higher foraging success. Another example for behavioral carryovers is inappropriate activity in streamside salamander larvae under predation by green sunfish: active individuals have higher feeding rates and develop faster in the absence of predators, but experience severe predation costs when sunfish are present (Sih et al. 2003).

Consequently, behaviors should always be studied in suites or packages, because behaviors might otherwise seem maladaptive in an isolated context. All of these points have created a surge of interest in animal personality among behavioral ecologists and led to various new discoveries. Most of these studies investigated personalities among nonsocial individuals. This is surprising, because some of the ecologically most dominant and successful animals live in social groups.

### **Collective personality**

Structured groups of related individuals such as the social insect societies are so highly developed that a whole colony can be compared to a single organism with the queen as the reproductive organ and her workers as the extended phenotype (Wheeler 1911). For that reason, insect colonies are often called “superorganisms” (Oster and Wilson 1978; Hölldobler and Wilson 2009). As William Morton Wheeler had already stated in 1911, every colony has

its own identity: some colonies might be more aggressive, while others are better foragers. Colonies of harvester ants differ in their foraging activity and regulation, which in turn may influence colony survival and reproduction (Gordon et al. 2011). Because selection also acts on the group level in social insects (Korb and Heinze 2004), these differences in collective personalities should therefore result in profound fitness consequences for the whole colony. However, only few studies investigated fitness consequences of collective personalities so far, e.g. honeybee colonies with a stronger defensive response and higher foraging activity gained more weight (fitness measure) and had higher winter survival (Wray et al. 2011).

### **Benefits of within-group variation in behavior**

Another aspect that has rarely been studied is the importance of within-group variation in behavior. Although there is much theoretical work on the benefits of within-group variation, actual empirical evidence is surprisingly scarce. A recent study on the social spider, *Anelosimus studiosus*, is one of the few examples that actually demonstrate how within-group behavioral variation influences fitness: female spiders experienced increased fitness when their groups contained behaviorally dissimilar individuals, i.e. aggressive and docile types (Pruitt and Riechert 2011). Nonacs and Kapheim (2007, 2008) demonstrated mathematically that interactions of genetic diverse individuals can result in mutualistic benefits that will maintain genetic diversity, though directional selection and genetic drift should reduce it. They termed this process “social heterosis”. Further, diverse groups can avoid or at least moderate trade-offs (e.g. costs of high aggression) that solitary individuals face. Finally, within-group variation should contribute to the division of labor and lead to a more efficient task allocation (Oster and Wilson 1978; Wilson 1987; Hölldobler and Wilson 1990; but see Dornhaus 2008).

### **Division of labor**

Division of labor has been recognized as one of the cornerstones of the ecological success of insect societies (Wilson 1987). It can be described by two principal components: (i) simultaneous performance of multiple tasks by different individuals and (ii) individual specialization on distinct activities over a certain timeframe (Oster and Wilson 1978; Robinson 1992). The most basic form of division of labor is that between the reproductive queen and the usually sterile workers. The workers are then further divided into exterior workers that forage, scout and patrol outside of the nest, and interior workers that take care of the brood. There are two common patterns of division of labor: temporal polyethism and

morphological polyethism (for a review on division of labor see Beshers and Fewell 2001). Temporal polyethism describes task allocation according to age: workers start working inside the nest and take care of the brood, when they grow older they progress to more risky tasks outside the nest like foraging or scouting (Seeley 1982; Calabi et al. 1983). In many social insects tasks are further allocated according to body size, which is called morphological polyethism. Leafcutter ants are comprised of different morphological castes that take over distinct roles in the colony (from smallest to largest workers): “gardener-nurses, within-nest generalists, forager-excavators, and defenders” (Wilson 1980). Further, division of labor and individual worker behavior are regulated by genetic factors, physiology, experience, social interactions, spatial dynamics and/or the environment (Robinson 1992; Franks and Tofts 1994; Fénelon et al. 1996; Gordon 1996; Ravary et al. 2007).

But how does this task allocation work exactly? According to the response threshold model, a worker will only perform a specific task if the stimulus exceeds her threshold (Bonabeau et al. 1996). A high variation in response thresholds is assumed to lead to an efficient self-organized task allocation. Myerscough and Oldroyd (2004) showed in computer simulations that colonies with uniform task thresholds are poor in reacting to changing colony needs, whereas colonies with individuals of differing task thresholds are able to respond fast and effectively. Indeed, empirical studies on the thermoregulation of honeybee colonies confirmed that diversity in worker response thresholds leads to stable brood nest temperatures despite environmental temperature fluctuations (Jones et al. 2004).

All these features make social insects and ants in particular an ideal model organism to study the influence of individual and colony level variation on group performance. Before I come to the goals of my dissertation, I will shortly introduce my study species.

### **The *Temnothorax* and *Leptothorax* genera**

The ant genera *Temnothorax* and *Leptothorax* belong to the myrmicine tribe Formicoxenini which is a frequently used model system for the study of social evolution and life history in particular (see Heinze 2006 for a recent review). Workers of both genera are quite small (1.7 - 4.5 mm) and so are their colonies, which contain on average between a few dozen and a few hundred individuals. These ants inhabit preformed cavities in acorns, hickories, sticks or rock crevices from cold habitats like tundra-forests to dry habitats like the semi-desert (Radchenko 2004).

*Temnothorax longispinosus* is a facultatively polygynous (a colony can contain multiple queens) ant species that inhabits mixed deciduous forests in the northeastern United

States and Canada. Although a single colony can inhabit several nest sites (i.e. a colony can be polydomous), selection is presumed to act on the nest level in this species (Herbers and Stuart 1996). Studies on its social organization demonstrated division of labor and revealed that although *T. longispinosus* is a monomorphic species, polyethism was related to polymorphism: foragers were larger than interior workers (Herbers 1983). Both the nest level selection and the demonstration of division of labor make this species a good candidate to study individual worker personality and the fitness consequences of its variation for the colony. In the last decade, *T. longispinosus* has received a lot of scientific attention owing to its coevolutionary arms race with the parasite slavemaking ant, *Protomognathus americanus* (e.g. Foitzik et al. 2001, 2009). As an obligatory slavemaker, *P. americanus* has to constantly replenish its slave working force by raiding *Temnothorax* ant colonies and stealing their brood. The slaves take over all the tasks in the nest, while the workers of the slavemaker do only one thing: they search for and attack host colonies. The interactions between parasite and host colonies are often highly violent and result in many mutilations and deaths (Foitzik et al. 2001, Brandt and Foitzik 2004). The parasite affects not only the life history, social structure and density of its host (Foitzik et al. 2009), but also its behavior (Pamminer et al. 2011). However, the host colonies have evolved counter adaptations to deal with the increasing threat. Just as Spartacus did, *T. longispinosus* slaves revolt against their slavemaking oppressors and kill the parasite's brood (Achenbach and Foitzik 2009). Studying the behavioral consequences of this parasite-host interaction could add valuable knowledge to the field of animal personality.

*Temnothorax nylanderi* is a common ant species in Central Europe and can reach densities of 10 nests per m<sup>2</sup> (Heinze et al. 1996). In contrast to *T. longispinosus*, established *T. nylanderi* colonies are strictly monogynous (Buschinger 1968). Only nest foundations can temporarily contain several queens (pleometrotic founding). Nest site limitation frequently leads to the fusion of unrelated colonies, but queen-queen aggression leads invariably to the reduction in queen number to one (Foitzik and Heinze 1998). If empty nests are available, *T. nylanderi* colonies occasionally split into several nest sites during summer (seasonal polydomy; Foitzik and Heinze 2001). Albeit they are not parasitized by slavemaking ants, intraspecific parasitism by unrelated founding queens takes place (Foitzik and Heinze 2000).

*Leptothorax acervorum* is the most common species of the genus *Leptothorax*. Colonies of this Holarctic ant species are extremely cold tolerant (up to – 40°C) and usually contain 80-200 workers with 1-10 queens (Seifert 2007). Workers of this species frequently lay unfertilized eggs (that develop into males) and thereby contribute to the reproductive

output of the colony. While reproductive conflicts among queens in polygynous colonies do not result in overt aggression (they lead to egg cannibalism), aggression among workers is common (Bourke 1991). Therefore, it would be intriguing to study the effects of ovarian development on worker personality and division of labor in this species.

## Goals

My dissertation research aims to identify how behavioral variation between and within colonies of *Temnothorax* ants influences colony performance. We consider both productivity in the field (under competition) and in the laboratory (no competition) to investigate what kind of behavior (e.g. aggression and exploration) is favored in a specific situation. If within-group variation in behavior is beneficial, colonies with more diverse workers should be more productive. To find out whether *T. longispinosus* colonies indeed show a “colony personality” we analyzed whether observed behavioral variation between colonies was consistent over time and different contexts.

We then investigated how natural selection and phenotypic plasticity shape behavior of *T. longispinosus* colonies in a natural situation. Mean colony aggression was positively correlated with population density (Modlmeier and Foitzik 2011) and encounters with slavemaking workers generated an increase in host aggression for several days (Pamminger et al. 2011). Phenotypic plasticity would be one possible explanation for the increased aggression. Alternatively, aggressive colonies might be able to outcompete less aggressive ones. To disentangle genetically-fixed base aggression from plastic effects in response to ecological factors (density, parasites), we conducted a field manipulation in a full factorial design. Owing to an early mating flight of the slavemaking ant *P. americanus*, we were able to examine how these parasites affect the productivity of *T. longispinosus* host colonies in the field and if higher host aggression helps against parasite invasion. During a slavemaker founding event, higher host aggression should be favored, because aggressive workers can immobilize (or even kill) the intruding *P. americanus* queen, while other workers evacuate the queen and the brood.

In order to analyze how morphological and physiological characteristics influence individual worker personality and division of labor in *L. acervorum* ants, we combined classical social insect methodology with the recent animal personality framework. Therefore, we study behaviors in the nest setting (e.g. foraging and activity) and in standardized experiments (e.g. aggression and exploration) and relate them to body size and ovarian development. Ovarian development and aggression are particularly interesting, because *L.*

*acervorum* workers can lay unfertilized eggs and often interact aggressively with nestmates (Bourke 1991).

In the last chapter of my dissertation, we investigated the emergent actions of ant colonies as a whole, i.e. the collective personality and not the individual personalities. Analyzing colony-level behaviors can give us insights into group-level selection and allows us to examine how efficient colonies perform cooperative tasks. To investigate the fitness consequences of collective personalities, we examined four specific colony-level behaviors in *T. nylanderi* colonies (nest relocation, nest defense, corpse removal and nest reconstruction) and their behavioral syndrome structure with particular regard to their relationship with productivity and immune defense. The ability to defend the colony against intruders, to repair the nest, to remove infected corpses and if necessary to relocate the colony to a new nest should strongly affect colony survival and productivity. Finally, we questioned whether behaviors that increase the exposure of workers to micro-parasites are linked to higher immune defense levels.

## **Chapter I: Productivity increases with variation in aggression among group members in *Temnothorax* ants**

This chapter is based on an original research article accepted in *Behavioral Ecology* in May 2011.

**Modlmeier AP**, Foitzik S. 2011. Productivity increases with variation in aggression among group members in *Temnothorax* ants. *Behav Ecol.* 22: 1026-1032



Close-up of a *Temnothorax longispinosus* worker.  
© Andreas Modlmeier

My contributions to this paper are: (i) experimental design, (ii) collection of data and statistical analysis, (iii) figure development, (iv) writing a first draft and revising it until completion, and (v) responding to reviewer comments

## Abstract

Social insect societies are characterized not only by a reproductive division of labor between the queen and workers but also by a specialization of workers on different tasks. However, how this variation in behavior or morphology among workers influences colony fitness is largely unknown. We investigated in the ant *Temnothorax longispinosus* whether aggressive and exploratory behavior and/or variation among nest mates in these behavioral traits are associated with an important fitness measure, that is, per worker offspring production. In addition, we studied how body size and variation in size among workers affect this colony fitness correlate. First, we found strong differences in worker body size, aggression, and exploration behavior among colonies. Most notably, intracolony variance in aggression was positively correlated with per worker productivity, suggesting a selective advantage of colonies with a higher variability in worker aggression. Because ant colonies in dense patches were both more aggressive and more productive, we cannot exclude the possibility that higher productivity and greater variability in aggression could both be results of good habitat quality and not causal influences on one another. This study suggests that social insect societies with stronger behavioral variation among nest members, and possibly a more efficient task allocation, are more productive in the field.

**Keywords:** aggression, competition, division of labor, fitness, habitat quality, natural selection, personality, social insects, *Temnothorax*



## Background

The ecological success of insect societies can be largely attributed to their division of labor (Wilson 1987; Hölldobler and Wilson 1990). The primary division of labor is that between the reproductive caste—the queen—and the usually sterile workers. Tasks are further subdivided among the workers, often resulting in morphological or temporal castes (Hölldobler and Wilson 1990). Division of labor is characterized by 2 principal components: 1) simultaneous performance of multiple activities by different individuals and 2) the specialization of individuals on one activity over some meaningful time frame (Oster and Wilson 1978; Robinson 1992). Why this specialization evolved is still unclear. One general assumption was an increased individual efficiency through task specialization. Surprisingly, this was not supported by empirical data from *Temnothorax* ants, which showed that specialization does not necessarily increase individual efficiency (Dornhaus 2008). As Chittka and Müller (2009) pointed out, empirical evidence remains scarce that division of labor and task specialization increase colony efficiency. Therefore, studies on the intracolony variation in behavior and its fitness benefits for the colony are needed to understand the evolution and maintenance of division of labor, one of the most characteristic traits of social insects.

According to the response threshold models (reviewed in Beshers and Fewell 2001), division of labor and its individual task specialization are generated by variation in response thresholds among workers within a colony. Workers will perform a task if the corresponding stimulus exceeds their internal threshold (Bonabeau et al. 1996). Specialists are therefore workers who have a lower threshold for a specific task and a higher threshold for other tasks. Consequently, task specialists represent distinct behavioral types of a colony. The term “behavioral type” has not been used in the social insect literature but has been coined to describe consistent multidimensional behavioral variants in nonsocial animals. In recent years, animal species that show this consistent behavioral variation have been described to exhibit personalities or behavioral syndromes (Sih et al. 2004). Studying behavioral variation in relation to reproductive success can yield valuable insights into the direction and intensity of natural selection on behavioral traits. According to a recent meta-analysis, personality dimensions such as aggression, exploration, and boldness were found to have important fitness consequences in many different species (Smith and Blumstein 2008). We expect to find similar effects in ants where consistent variation in behavior and social organization of colonies can have a genetic basis (Stuart and Page 1991; Ross and Keller 1998). There is, however, an important difference between social insects and solitary species. In contrast to solitary animals, natural selection in social insects can also act on the colony level (Korb and

Heinze 2004). The importance of group selection for the evolution of eusociality is currently controversial (Nowak et al. 2010; Okasha 2010). In addition to between-colony variation in behavior, resulting in behavioral types of colonies, insect societies could also differ in the variation within the colony, that is, the number and distinctness of behavioral types present within a colony.

Many studies on animal personalities include variation in exploration behavior (Verbeek et al. 1994; Dingemanse and Réale 2005). Although exploratory animals have a higher chance to discover food sources, they also take higher risks and have higher metabolic rates (Careau et al. 2008). As a consequence of their more active and exposed lifestyle, they consume more energy and are prone to predation. Another personality dimension, aggression, plays a fundamental role in nest defense and in the competition for resources or nest sites (Collias 1944; Kontiainen et al. 2009). The effect of worker aggression on colony fitness under field conditions has not been explored so far. Although aggressive behavior may help in acquiring and defending resources, it involves apparent costs, including higher metabolic rates and the risk of injury or death (Holway et al. 1998; Gobin et al. 2003). Furthermore, time and energy spent fighting cannot be used for other important social interactions, such as brood care.

Social insects provide the opportunity to study the benefits of behavioral variation for animal groups. In insect societies, specific individuals can become allocated to certain tasks, leading to a high variance of behavioral types among nest mates and consequently to a strong division of labor. This variation could be particularly beneficial when behavioral uniformity is costly. For example, under high-density conditions, colonies with a higher variability in aggressive behavior could be favored by natural selection: whereas the aggressive behavioral types defend the colony, less aggressive nest mates care for the brood. We therefore predict that high variation in worker behavior should improve colony productivity. We tested this prediction in the ant species, *Temnothorax longispinosus*, which was already known to exhibit a clear division of labor (Herbers and Cunningham 1983). We investigated whether aggressive or exploratory behavior and variation among nest mates in these traits were associated with a measure of fitness (i.e., per worker productivity of a colony). Brood production in *Temnothorax* ants is highly synchronized, and we collected ant colonies shortly before the emergence of the new workers and sexuals. Hence, the observed offspring production should be a reliable assessment of the total annual production (Headley 1943; Kipyatkov 1993).

Aside from behavioral variation, morphological variation may also contribute to the productivity of ant colonies. First, task performance can be linked to the body size of workers. One of the most extreme cases of size variability among workers (by the factor 6, based on head width measurements) can be found in leafcutter ants of the genus *Atta*. In these fungus-growing ants, smaller workers focus on the maintenance of the fungus garden, mid-sized workers degrade leaves and carry the fragments back to the nest, and the largest worker castes are involved in cutting of the vegetation (Wilson 1980b). Maintaining this variability in worker sizes within colonies of polymorphic ant species is not only important for worker survival (Billick and Carter 2007) but also for brood production (Porter and Tschinkel 1985; Billick 2002). Second, worker size in social insects can be associated with task efficiency, described as superior performance under normal conditions (Couvillon and Dornhaus 2010). Support for this hypothesis comes from bumblebees where larger workers collect more nectar per unit time (Goulson et al. 2002; Spaethe and Weidenmüller 2002). In contrast, smaller workers are more resistant to starvation and are presumably cheaper to produce (Couvillon and Dornhaus 2010). Despite being generally monomorphic, workers of our focal ant *T. longispinosus* vary in size by the factor 1.3 in head width within a colony (Figure 1a), and this variation is related to their behavioral caste (Herbers and Cunningham 1983). It is unclear whether size also determines task efficiency and ultimately colony fitness in ant species with monomorphic workers and a much lower variation in worker body size. Therefore, the second part of this study will investigate whether worker body size and its variation influence colony productivity.

In social insects, per worker productivity was shown to decrease with colony size (“Michener’s paradox,” Michener 1964), and this relationship was thought to oppose the evolution of large insect societies. However, recent studies found varying relationships between colony size and productivity, including positive, negative, and no clear relationships (for references, see Dornhaus et al. 2008). Hence, colony size, that is, worker number, can certainly influence colony fitness. Furthermore, productivity is not only affected by colony characteristics but also by ecological conditions, such as food availability and competition. In ants, nest density can reflect local habitat quality and is often associated with intraspecific competition (Hölldobler and Wilson 1990). In dense patches, foraging ants frequently encounter intraspecific competitors, which can cause adaptive increases in the aggression level (Thomas et al. 2007; Van Wilgenburg et al. 2010). Therefore, we analyzed whether nest density was associated with higher aggression levels and/or a higher productivity in the nonterritorial *T. longispinosus* ants.

The central hypothesis of this study was that behavioral and morphological variation among group members contributes to fitness in ants. Specifically, we test these 2 predictions: 1) Intracolony variation in aggressive and exploratory behavior improves colony productivity and 2) intracolony variation in worker size improves colony productivity.

## Materials and methods

### Study system

Our study species, *T. longispinosus*, lives in mixed deciduous forests across the northeastern United States and inhabits preformed cavities in acorns, hickory nuts, and twigs. Although these ants are facultatively polygynous and polydomous, most colonies at our study site in New York contain a single queen and a single nesting unit (Foitzik et al. 2004). Only about 10% of all nests have several queens (data from Foitzik et al. 2009 based >750 nests). Polydomy is relatively rare as genetic analyses (Foitzik et al. 2004) showed that 10.7% of all colonies were polydomous, each consisting of 2 or 3 subunits. We did not genotype all colonies (and the surrounding nests), so that we cannot rule out that some of the colonies included in our analyses were indeed polydomous. We decided to analyze the data on the level of the local nest unit because selection was suggested to act on the nest level in this species (Herbers and Stuart 1996).

Colonies were collected in oak-hickory forests at the Huyck Preserve, Albany County, NY (USA), between 10 AM and 5 PM in early August 2009, at a time of the day during which foraging is ongoing. *Temnothorax* ants forage solitary, and less than 10% of all workers leave the nest for foraging at any one time (Foitzik S, personal observation). We estimate that we have missed 1–5 workers per nest depending on its size. However, this fraction should be similar for all colonies as we only collected nests during the active foraging period. At the time of collection, ant colonies contained sexual and worker brood shortly before emergence from the pupae. Ant colonies were censused at the field station within days of collection and transported to the laboratory in Munich (Germany), where they were transferred to artificial nests in 3-chambered plastic boxes (9.5 × 9.5 × 2.7 cm) with a moistened plaster floor. Ants were fed twice weekly with honey and pieces of dead crickets.

We included only well-established monogynous colonies with at least 20 workers in all experiments ( $n = 39$  colonies, mean worker number = 44.69, standard deviation (SD) = 20.83). Thereby, we reduced the impact of colony size and eliminated potential effects of queen number on worker behavior or per capita productivity. All behavioral experiments were

conducted by the same person (A.P.M.) in the laboratory in Munich between 31 August 2009 and 17 October 2009. Behavioral experiments were started 2 weeks after collection in the field.

### **Per capita productivity**

We used total biomass production divided by the number of workers (per capita productivity) as a measure of fitness. In *Temnothorax* ants, the different castes, that is, queens, workers, and males, vary in size and biomass. The average dry weight of *T. longispinosus* queens is 873.4  $\mu\text{g}$ , whereas workers and males weigh only 216.6 and 231.1  $\mu\text{g}$ , respectively (Foitzik et al. 2004). Hence, total offspring production of a colony was calculated by multiplying the total number of worker, male, and queen pupae with the respective mean values of dry weight for adult workers, males, and virgin queens as previously determined for colonies collected at the same site (Foitzik et al. 2004). Early-stage pupae, so-called prepupae, whose caste could not be reliably determined, were included in the analyses as worker pupae. Per capita productivity was calculated by dividing the total dry mass production of a colony by the number of adult workers residing in the ant nest.

### **Competition and nest density in the field**

To investigate how ecological conditions (especially competition) affect ant behavior, we analyzed colony density in the field. Thirty-five of the 39 colonies were taken from  $6 \times 3$  m study plots, which were carefully searched for all ant colonies. The positions of all *T. longispinosus* colonies within these plots were recorded. In addition, we mapped the position of all colonies of the slave-making ant, *Protomognathus americanus*, which contained enslaved *T. longispinosus* workers. The latter colonies were included in the evaluation of nest density because *Temnothorax* slaves take over the foraging task in these colonies and thus directly compete with foragers from unparasitized colonies. *Temnothorax* workers are only 2–3 mm in length. For the congeneric species, *T. nylanderi*, the mean foraging distance was determined to be below 20 cm (Heinze et al. 1996). We therefore were interested in nest density in the immediate vicinity of each nest. The  $6 \times 3$  m study plots were divided into 72 squares of  $50 \times 50$  cm. Local density was subsequently determined for each colony by counting the number of ant nests in the same square and in the 5 closest squares inside the study plot. We used squares instead of density within a given distance because thereby we avoided extrapolation for colonies located at the plot border. We estimated the nest density for

the 4 colonies outside of the plots by using average densities per 1.5 m<sup>2</sup> of the closest study plots.

### **Order of experiments**

We randomly selected 10 workers per colony that were used throughout all experiments. Workers were tested for exploration first and followed by an aggression test. In-between experiments, workers were kept separately in Petri dishes (diameter 33 mm and height 11 mm) with a wet piece of paper for approximately 1 h. After the aggression experiment, workers were frozen for the morphological analysis.

### *Aggression experiments*

In order to estimate mean aggression and intracolony variance in aggressive behavior, 10 workers per colony were tested separately. Each worker was transferred to a small circular arena (diameter 12 mm and height 3 mm) with a freshly defrosted dead conspecific from 1 of 8 queenright colonies from a population in West Virginia. Because *Temnothorax* colonies contain on average less than 50 workers, we had to use opponents from several colonies. Variation in chemical profiles of opponents from different colonies could lead to variance in aggression. To eliminate this possibility, we used for a single test colony always dead workers from the same colony. By using a dead adversary, any effects of the opponents' behavior could be excluded (Crosland 1990; Roulston et al. 2003). However, to ensure that experiments with dead opponents reflect aggression of workers also in live encounters, we performed additional experiments with laboratory *T. longispinosus* colonies. We marked 8 workers per nest and tested them individually 3 times against a single-living conspecific worker from a different colony to get a good estimate of their average aggression. Subsequently, each worker was tested a fourth time, but the opponent in this test was a dead conspecific worker. In-between experiments, marked workers were returned to their original colony for 1 week. These experiments confirmed that individual aggression against dead ants significantly correlates with the average aggression of repeated tests against living ants (Spearman's rank correlation:  $r_s = 0.45$ ,  $P < 0.001$ ,  $n = 52$  workers).

We calculated the proportion of aggressive behavior of a worker by recording its behavior every 20 s over 5 min and then dividing the number of aggressive interactions by the total number of observations. Aggressive interactions included mandible opening (threat display), biting, dragging, carrying, and stinging. For further analysis, we used mean and SD over 10 different workers from each colony as a measure of the level and the variation in

behavior within the colony. We used the SD as a measure of the behavioral variation within colonies instead of the variance because the latter is more prone to be influenced by outliers.

If a dead opponent was not attacked, we reused it in up to 4 trials. We tried to ensure that the chemical profile of these reused opponents did not change. First, we did not reuse opponents that were physically attacked by biting and stinging. Second, each experiment lasted only 5 min plus approximately 3 min for preparation. Thus, dead opponents were kept less than 40 min outside of the freezer. Third, we always tested the 10 workers of a colony on a single day. Every colony started with a new (unused) opponent and had therefore the same chance of being tested against unused workers. We wiped the arena carefully with ethanol after each trial to eliminate potential residual odors.

### *Exploration experiments*

We used a multichamber setup to test exploratory behavior. The experimental setup consisted of a central chamber (diameter 29 mm and height 3 mm) that was connected to 8 equally sized side chambers through 8 corridors (length 32 mm and width 7 mm). Each of the 8 chambers contained a different, unknown but chemically distinct object, mainly spices: dried pieces of spruce needles, oregano, caraway, thyme, rosemary, sage, chamomile, and savory. When an ant was released in the central chamber, we recorded for 5 min how often it would antennate these unknown objects. A single antennation event started when the worker touched one of the unknown objects with its antennae. If the contact was interrupted for more than 1 s, a new antennation was counted. We used the mean and SD of the number of antennations over the 10 different workers from each colony as a measure of the level and the variation in exploratory behavior within each colony. After each experiment, we entered new objects and wiped the arena with ethanol.

We used the term “exploratory behavior” following Verbeek et al. (1994) and R  ale et al. (2007), who classified both the exploration of novel environments and of novel objects as exploratory behavior. We chose to use the number of antennations as a measure of exploratory behavior because a possible alternative measure, the number of chambers that an ant investigated, would be strongly influenced by the activity level of the ant. Individuals ants could just by running around enter many chambers and not because they behave very exploratory. In contrast, antennation is a better measure of interest for a novel object.

## **Worker size**

In order to estimate mean worker size and variation in a colony, the head width of 10 workers per colony was measured to the nearest 0.01 mm with an ocular micrometer. Head width is the most commonly used character to estimate worker size in ants (Wilson 1980a; Herbers and Cunningham 1983).

## **Statistical analyses**

We used Kruskal–Wallis tests to check for differences in behavior or morphology between colonies. In order to analyze which parameters were associated with behavioral or morphological differences among colonies, we performed stepwise multiple regressions with mean colony aggression or exploration as dependent variables. The multiple regression model was selected in a backward stepwise process, first including all explanatory variables and then removing factors until the highest adjusted  $r^2$  value was reached. Explanatory variables for both behavioral traits were colony size ( $N$  of adult workers), mean colony head width, SD of colony head width, relative colony productivity (dry number production per worker), and nest density. Additionally, we included mean exploratory behavior as an explanatory variable for mean aggression and accordingly mean aggression as an explanatory variable for exploratory behavior.

Finally, we wanted to investigate if variation in aggression, exploration, and body size can explain the analyzed fitness component, that is, the relative productivity of a colony. Therefore, we performed a second backward stepwise multiple regression but this time with the relative productivity as the dependent and the number of adult workers, mean colony head width, SD of colony head width, mean colony aggression, SD of colony aggression, mean colony exploration, SD of colony exploration, and nest density as explanatory variables. For the regression analyses, normality of residuals was tested with Kolmogorov–Smirnov tests ( $P > 0.2$ ). Homogeneity of variances was checked graphically by plotting the residuals against the values of the explanatory variables.

## **Results**

Colonies differed strongly in worker size, aggression, and exploration behavior (Kruskal–Wallis tests; worker size:  $H_{38,390} = 170.28$ ,  $P < 0.0001$ , Figure 1a; aggression:  $H_{38,390} = 78.66$ ,  $P < 0.0001$ , Figure 1b; and exploration:  $H_{38,390} = 69.56$ ,  $P < 0.002$ , Figure 1c).

Next, we explored colony-level variation in behavior and morphology. The final regression model ( $F_{2,36} = 4.97$ ;  $P < 0.013$ ;  $r^2_{\text{adjusted}} = 0.17$ ; Supplementary Table S2b), which explained



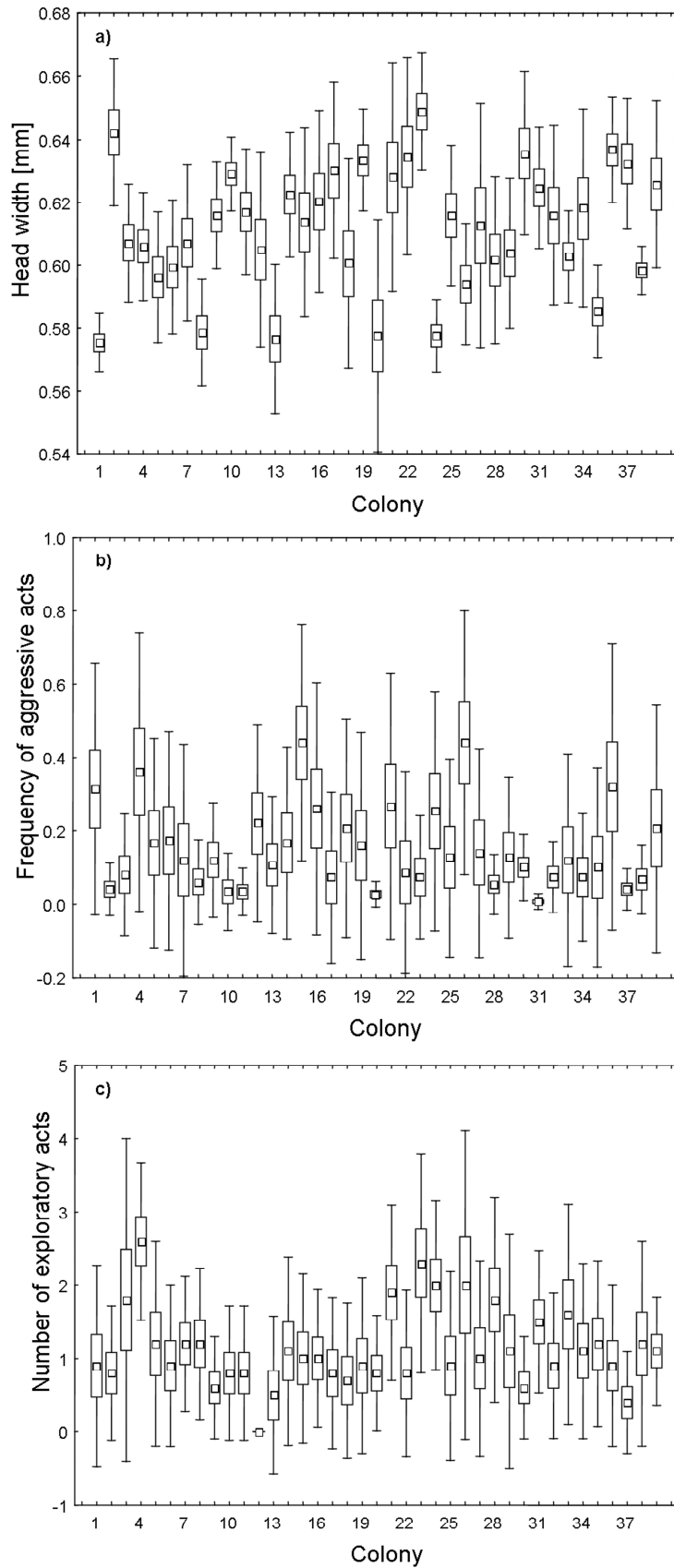
most of the differences in mean aggression among colonies, included nest density ( $b = 0.388$ ,  $P < 0.014$ ) and mean exploration ( $b = 0.217$ ,  $P = 0.152$ ). Colony aggression increased with nest density (Figure 2). Differences in mean colony exploration could not be explained by any of the independent factors, including the nest density, colony size, head width, aggression, and productivity. The best regression model (Supplementary Table S3b) included only mean aggression and SD of head width and was not significant (Stepwise multiple regression:  $F_{2,36} = 2.65$ ;  $P < 0.09$ ;  $r^2_{\text{adjusted}} = 0.08$ ).

Colony productivity was positively associated with the SD of aggression (backward stepwise multiple regression:  $b = 0.544$ ,  $P = 0.029$ ; Figure 3a) and the number of neighboring colonies ( $b = 0.487$ ,  $P = 0.004$ ; Figure 3b, Table 1). Hence, colonies with a higher SD in aggression and in denser areas showed a higher relative productivity.

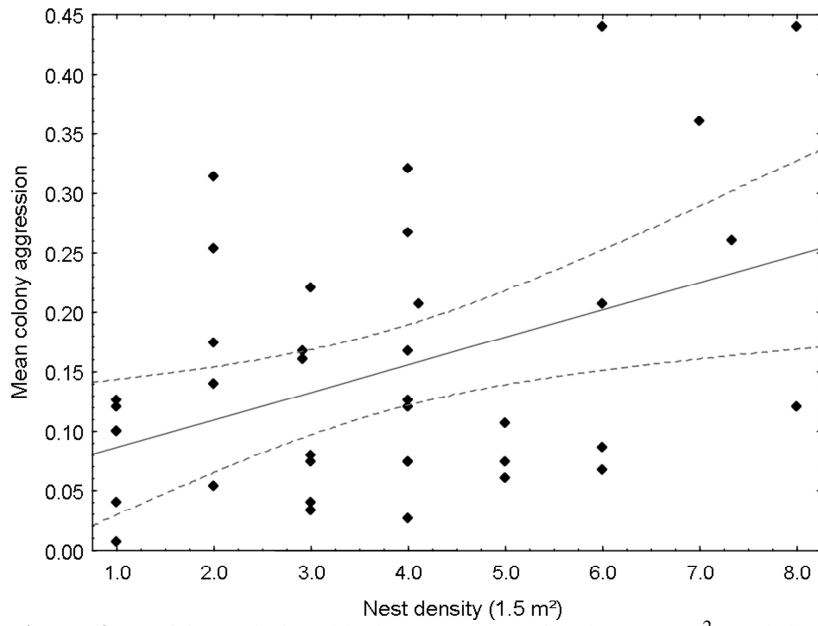
**Table 1.** Results of the best multiple regression model on the relative colony productivity (annual production in dry weight/number of workers) as the dependent variable.

<b>Explanatory variable</b>	<b>Beta</b>	<b>P value</b>
Mean colony aggression	-0.350	0.166
Standard deviation colony aggression	0.544	<b>0.029</b>
Mean colony exploration	-0.319	0.087
Standard deviation colony exploration	0.195	0.279
Nest density (1.5 m <sup>2</sup> )	0.487	<b>0.004</b>
Standard deviation of colony head width	-0.166	0.271

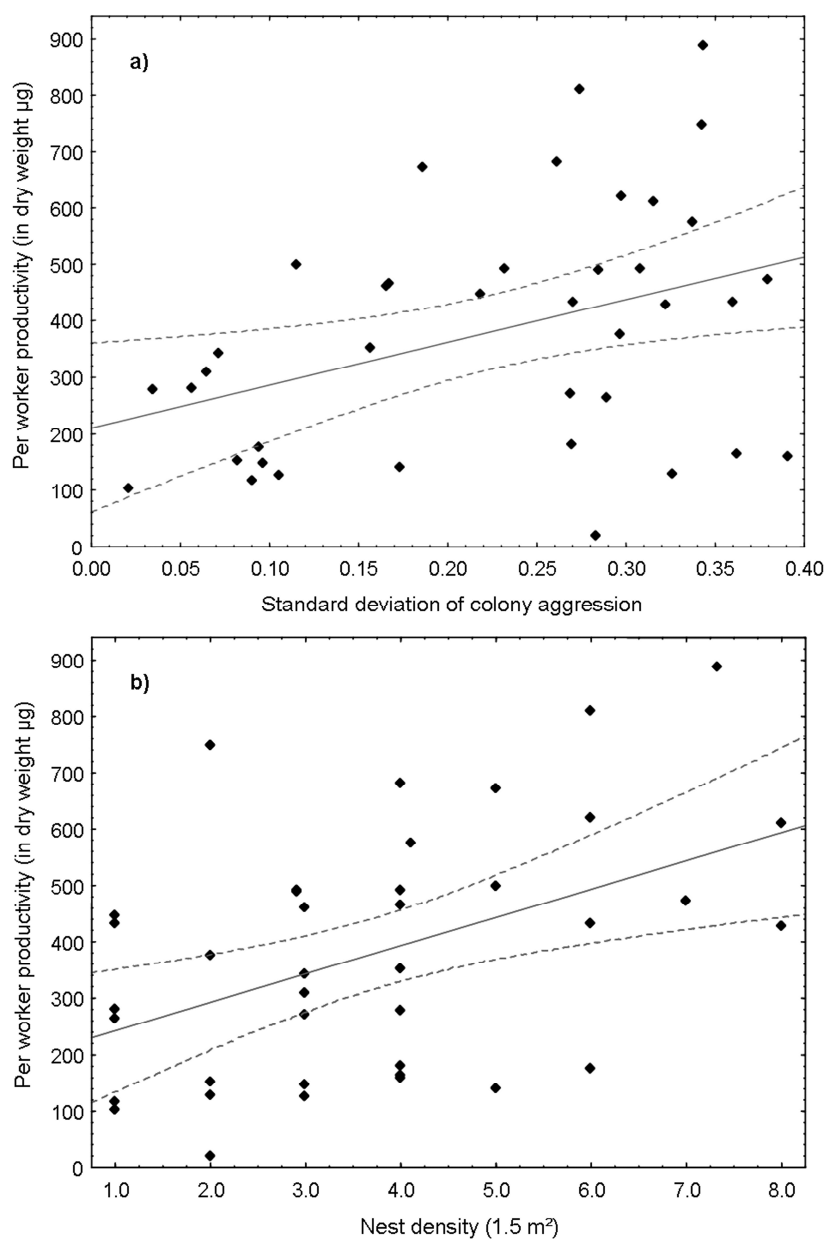
The model was selected in a backward stepwise process, first including all explanatory variables and then removing factors until the highest adjusted  $r^2$  value was reached ( $F_{6,32} = 3.42$ ;  $p < 0.011$ ;  $r^2_{\text{adjusted}} = 0.28$ ). In the original model (Supplementary Table S1) were the following explanatory variables included: the number of adult workers, mean colony head width, SD of colony head width, mean colony aggression, SD of colony aggression, mean colony exploration, SD of colony exploration, and nest density (1.5 m<sup>2</sup>). Colony behavior was analyzed by testing 10 ant workers per colony separately in standardized experimental set-ups. A total of 39 colonies were included. Significant P values are shown in bold.



**Figure 1.** Differences in a) mean colony head width, b) aggressive behavior and c) exploratory behavior among 39 colonies of *Temnothorax longispinosus* from a study site in upstate NY, USA. 10 workers per colony were tested. Results are shown as means with the box showing SE and the brackets showing SD.



**Figure 2.** Positive relationship between nest density (1.5 m<sup>2</sup>) and the mean colony aggression in 39 tested colonies of *Temnothorax longispinosus* from a study site in upstate New York, USA. Figure includes 95% confidence bands.



**Figure 3.** Positive relationship between the fitness component (per worker productivity) and (a) the SD of colony aggression and (b) nest density ( $1.5 \text{ m}^2$ ). Thirty-nine colonies of *Temnothorax longispinosus* from a study site in upstate New York, USA, were tested. Figures include 95% confidence bands.

## Discussion

The results of this study revealed that the productivity of ant colonies increased with intracolony variation in aggressiveness, which suggests a selective advantage of behavioral variation among group members. These colonies might be more productive because tasks such as nest defense and brood care are taken over by specialized workers with different thresholds. Whereas workers with a low threshold for aggressive responses engage in competitive interactions with other colonies, less aggressive social workers take care of the brood. Albeit variation in worker behavior or in other words in the division of labor is thought to be the basis for the ecological success of social insects, there are very few studies, if any, that show a direct relationship between behavioral variation and colony fitness. What has been shown is that social insect colonies that have a higher genetic diversity among workers show a higher productivity (Cole and Wiernasz 1999; Wiernasz et al. 2004), a better temperature regulation (Jones et al. 2004), and an increased resistance against disease infections (Tarpy and Seely 2006). These positive effects of genetic diversity were interpreted to be the result of a stronger variation in response thresholds in genetically diverse colonies. However, we do not expect strong differences in genetic variability between the *T. longispinosus* colonies tested here because all study colonies were monogynous and this species is also known to be invariably monandrous (Foitzik et al. 2004).

A common problem of laboratory experiments in behavioral ecology is that behaviors can differ between a field and a laboratory setting. Nevertheless, we argue that our laboratory observations, especially those concerning the behavioral differences among colonies, can be used to examine the results in an ecological and evolutionary perspective for several reasons: First, we found that the behavior of the laboratory colonies did not change over the duration of the experiments (August 2009 to October 2009). Moreover, we were able to show that the behaviors of *T. longispinosus* colonies (mean aggression and variability in aggression, Modlmeier AP, Foitzik S, unpublished data) are consistent for at least 4–5 months, with a new generation of workers emerging in between the behavioral experiments. Similarly, the aggressiveness of colonies and the number of aggressive workers remained constant in a study on the ant *Rhytidoponera confusa* (Crosland 1990). These findings support the general assumption of response threshold models that behavioral thresholds are fixed and have a genetic basis (Beshers and Fewell 2001). In theory, this could lead to intercolony variation in behavior and thus to personalities on a colony level. Nevertheless, the mechanisms that regulate response thresholds in ants are still poorly understood. Differences between individuals can depend on genotype, age, and experience (Page and Erber 2002). Workers that

are successful at one task might show a higher tendency to perform this task again compared with those that failed. Clonal same-aged workers of the parthenogenetic ant, *Cerapachys biroi*, that were allowed to capture prey continued to forage over weeks, whereas unsuccessful workers specialized on brood care and refrained from leaving the nest to forage (Ravary et al. 2007). Consequently, individual experience could also influence exploratory and aggressive behavior in *T. longispinosus*.

Our results show that ecological factors might influence worker behavior. Variation in aggression between colonies could be in part explained by density in the field as the mean aggressiveness of workers from a colony was positively correlated with nest density. In the invasive Argentine ants, contact to intraspecific competitors and fighting experience lead to elevated levels of aggression (Thomas et al. 2007; Van Wilgenburg et al. 2010). Hence, the generally much less aggressive and nonterritorial *T. longispinosus* ants might similarly react to frequent contacts with competitors by increasing the aggression level over longer time periods. In other words, a phenotypic plastic response to crowding at the population level might raise intraspecific aggression. Alternatively or in addition, only aggressive colonies might be able to compete in dense populations with many competitive encounters between neighboring colonies. Albeit *Temnothorax* ants do not defend territories (Heinze et al. 1996), less aggressive colonies might be outcompeted or evicted from their nest sites, which are a main limiting ecological parameter in the *T. longispinosus* population at our New York study site (Herbers 1986). Nest sites of these ants—acorns, hickory nuts, and twigs—decompose during the warm and wet summer months and become limited at the end of the season (Foitzik and Heinze 1998). *Temnothorax* colonies are forced to relocate their nests and might end up close to competing ant colonies. Natural selection could therefore lead to an occupation of high-density areas by aggressive colonies.

Interestingly, the relative productivity of colonies increased with nest density. If intraspecific competition is important in this system, we would have expected an association in the opposite direction namely that productivity decreases with the number of competitors in the vicinity. However, nest density can be a reliable indicator of good habitat quality, which has been shown both in birds (Bock and Jones 2004; Pérot and Villard 2009) and ants (Kaspari et al. 2000). If also true for our system, high habitat quality, that is, food availability, could lead both to a higher ant nest density and a higher productivity of colonies, resulting in the observed positive correlation between nest density and productivity. The main conclusion from this finding would be that habitat heterogeneity is more important for ant colony productivity than competition for food.

This finding also suggests an alternative scenario in which higher productivity and greater variability in aggression could both be results of good habitat quality and not the result of one factor directly influencing the other. Good habitat quality could lead to higher productivity and higher nest density. The latter could increase not only mean aggression but also intracolony variation in aggressiveness if foragers and scouts engaging in frequent aggressive encounters outside of the nest become more aggressive than ants inside the nest.

As our results suggest a selective advantage of behavioral variation, the question arises why some colonies vary more in their behavior than others. One source of behavioral variability could be intracolony genetic variation. *Temnothorax* queens are generally singly inseminated, but genetic diversity can arise in this species through facultative polygyny. In honey bees, behavioral variability is indeed associated with genetic diversity (Fewell and Page 1993; Page et al. 1995), and the fitness advantage of genetic diversity has been shown in colonies of harvester ants and honey bees, where multiple mating leads to higher productivity and resistance against diseases (Cole and Wiernasz 1999; Tarpay 2003; Wiernasz et al. 2004).

Neither variation nor mean worker body size was associated with per capita productivity. Accordingly, behavioral variability was associated with a measure of fitness, whereas morphological variation was not. Although earlier experiments showed an association between body size and division of labor in *T. longispinosus*, where larger workers were more likely to forage (Herbers and Cunningham 1983), we did not find evidence that morphological variation in this monomorphic species is adaptive.

We uncovered strong intraspecific differences among ant colonies. Colony-level variation was found in worker body size and also in behavior of workers in standardized tests. Similar to the observed personalities in nonsocial animals (Verbeek et al. 1994; Réale et al. 2000), insect colonies appear to exhibit clear behavioral differences with multiple dimensions. Colonies not only differed in a single behavioral parameter but at least in 2- exploration and aggression.

In conclusion, we demonstrate that mean colony aggression increases with nest density, which can be due to natural selection, phenotypic plasticity in behavior, or a combination of both. Regardless of the mechanism, our findings underline the importance of aggression for the reproductive success of animals (Kontiainen et al. 2009), especially under high-density conditions. Our result that colony productivity increases with nest density in the New York population of *T. longispinosus* indicates that habitat heterogeneity is more important than competition in this environment. Finally, the key finding of this study, the empirical link between intracolony variation in aggressiveness and per capita productivity,

suggests that behavioral variation, presumably leading to a strong division of labor, can increase the fitness of social insect colonies.

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### References

- Beshers SN, Fewell JH. 2001. Models of division of labor in social insects. *Annu Rev Entomol.* 46:413-440.
- Billick I. 2002. The relationship between the distribution of worker sizes and new worker production in the ant *Formica neorufibarbis*. *Oecologia.* 132:244-249.
- Billick I, Carter C. 2007. Testing the importance of the distribution of worker sizes to colony performance in the ant species *Formica obscuripes* Forel. *Insect Soc.* 54:113-117.
- Bock CE, Jones ZF. 2004. Avian habitat evaluation: should counting birds count? *Front Ecol Environ.* 2:403-410.
- Bonabeau E, Theraulaz G, Deneubourg JL. 1996. Quantitative study of the fixed threshold model for the regulation of division of labour in insect societies. *Proc R Soc Lond B.* 263:1565-1569.
- Careau V, Thomas D, Humphries MM, Réale D. 2008. Energy metabolism and animal personality. *Oikos.* 117:641-653.
- Chittka L, Müller H. 2009. Learning, specialisation, efficiency and task allocation in social insects. *Commun Integr Biol.* 2:151-154.
- Cole BJ, Wiernasz DC. 1999. The selective advantage of low relatedness. *Science.* 285:891-893.
- Collias NE. 1944. Aggressive behavior among vertebrate animals. *Physiol Zool.* 17:83-123.
- Couvillon MJ, Dornhaus A. 2010. Small worker bumble bees (*Bombus impatiens*) are hardier against starvation than their larger sisters. *Insect Soc.* 57:193-197.
- Crosland MWJ. 1990. Variation in ant aggression and kin discrimination ability within and between colonies. *J Insect Behav.* 3:359-379.
- Dingemanse NJ, Réale D. 2005. Natural selection and animal personality. *Behaviour.* 142:1165-1190.
- Dornhaus A. 2008. Specialisation does not predict individual efficiency in an ant. *PLoS Biol.* 6:2368-2375.
- Dornhaus A, Holley J-A, Pook VG, Worswick G, Franks NR. 2008. Why do not all workers work? Colony size and workload during emigrations in the ant *Temnothorax albipennis*. *Behav Ecol Sociobiol.* 63:43-51.
- Fewell JH, Page RE. 1993. Genotypic variation in foraging responses to environmental stimuli by honey bees, *Apis mellifera*. *Experientia.* 49:1106-1112.



- Foitzik S, Achenbach A, Brandt M, 2009. Locally adapted social parasite affects density, social structure, and life history of its ant hosts. *Ecology* 90:1195-1206.
- Foitzik S, Backus VL, Trindl A, Herbers JM. 2004. Ecology of *Leptothorax* ants: impact of food, nest sites and social parasites. *Behav Ecol Sociobiol.* 55:484-493.
- Foitzik S, Heinze J. 1998. Nest site limitation and colony take over in the ant, *Leptothorax nylanderi*. *Behav Ecol.* 9:367-375.
- Gobin B, Heinze J, Strätz M, Roces F. 2003. The energetic cost of reproductive conflicts in the ant *Pachycondyla obscuricornis*. *J Insect Physiol.* 49:747-752.
- Goulson D, Peat J, Stout JC, Tucker J, Darvill B, Derwent LC, Hughes WOH. 2002. Can alloethism in workers of the bumblebee, *Bombus terrestris*, be explained in terms of foraging efficiency? *Anim Behav.* 64:123-130.
- Headley AE. 1943. Population studies of two species of ants, *Leptothorax longispinosus* Roger and *Leptothorax curvispinosus* Mayr. *Ann Entomol Soc Am.* 36:743-753.
- Heinze J, Foitzik S, Hippert A, Hölldobler B. 1996. Apparent dear-enemy phenomenon and environment-based recognition cues in the ant *Leptothorax nylanderi*. *Ethology.* 102:510-522.
- Herbers JM. 1986. Nest site limitation and facultative polygyny in the ant *Leptothorax longispinosus*. *Behav Ecol Sociobiol.* 19:115-122.
- Herbers JM, Cunningham M. 1983. Social organization in *Leptothorax longispinosus* Mayr. *Anim Behav.* 31:759-771.
- Herbers JM, Stuart RJ. 1996. Multiple queens in ant nests: impact on genetic structure and inclusive fitness. *Am Nat.* 147:161-187.
- Hölldobler B, Wilson EO. 1990. *The Ants*. Cambridge, MA.: Harvard Univ. Press.
- Holway DA, Suarez AV, Case TJ. 1998. Loss of intraspecific aggression in the success of a widespread invasive social insect. *Science.* 282:949-952.
- Jones JC, Myerscough MR, Graham S, Oldroyd BP. 2004. Honey bee nest thermoregulation: Diversity promotes stability. *Science.* 305:402-404.
- Kaspari M, O'Donnell S, Kercher JP. 2000. Energy, density, and constraints to species richness: ant assemblages along a productivity gradient. *Am Nat.* 155:280-293.
- Kipyatkov VE. 1993. Annual cycles of development in ants: diversity, evolution, regulation. In: Kipyatkov VE, editor. *Proceedings of the colloquia on social insects*. St. Petersburg (Russia): Russian-speaking Section of the IUSI, *Socium.* 25-48.
- Kontiainen P, Pietäinen H, Huttunen K, Karell P, Kolunen H, Brommer JE. 2009. Aggressive Ural owl mothers recruit more offspring. *Behav Ecol.* 20:789-796.
- Korb J, Heinze J. 2004. Multilevel selection and social evolution of insect societies. *Naturwissenschaften.* 91:291-304.
- Michener CD. 1964. Reproductive efficiency in relation to colony size in Hymenopterous Societies. *Insect Soc.* 11:317-342.
- Nowak MA, Tarnita CE, Wilson EO. 2010. The evolution of eusociality. *Nature.* 466:1057-1062.
- Okasha S. 2010. Altruism researchers must cooperate. *Nature.* 467:653-655.
- Oster GF, Wilson EO. 1978. *Caste and ecology in the social insects*. Princeton: Princeton University Press.
- Page RE, Erber J, 2002. Levels of behavioral organization and the evolution of division of labor. *Naturwissenschaften.* 89:91-106.
- Page RE, Robinson GE, Fondrk MK, Nasr ME. 1995. Effects of worker genotypic diversity on honey bee colony development and behavior (*Apis mellifera* L.). *Behav Ecol Sociobiol.* 36:387-396.
- Pérot A, Villard M-A. 2009. Putting density back into the habitat-quality equation: case study of an open-nesting forest bird. *Conser Biol.* 23:1550-1557.

- Porter SD, Tschinkel WR. 1985. Fire ant polymorphism: the ergonomics of brood production. *Behav Ecol Sociobiol.* 16:323-336.
- Ravary F, Lecoutey E, Kaminski G, Châline N, Jaisson P. 2007. Individual experience alone can generate lasting division of labor in ants. *Curr Biol.* 17:1308-1312.
- Réale D, Gallant BY, Leblanc M, Festa-Bianchet M. 2000. Consistency of temperament in bighorn ewes and correlates with behaviour and life history. *Anim Behav.* 60:589-597.
- Réale D, Reader SM, Sol D, McDougall PT, Dingemans NJ. 2007. Integrating animal temperament within ecology and evolution. *Biol Rev.* 82:291-318.
- Robinson GE. 1992. Regulation of division of labor in insect societies. *Annu Rev Entomol.* 37:637-665.
- Ross KG, Keller L. 1998. Genetic control of social organization in an ant. *Proc Natl Acad Sci USA.* 95:14232-14237.
- Roulston TH, Buczkowski G, Silverman J. 2003. Nestmate discrimination in ants: effect of bioassay on aggressive behavior. *Insect Soc.* 50:151-159.
- Sih A, Bell A, Johnson JC. 2004. Behavioral syndromes: an ecological and evolutionary overview. *Trends Ecol Evol.* 19:372-378.
- Smith BR, Blumstein DT. 2008. Fitness consequences of personality: a meta-analysis. *Behav Ecol.* 19:448-455.
- Spaethe J, Weidenmüller A. 2002. Size variation and foraging rate in bumblebees (*Bombus terrestris*). *Insect Soc.* 49:142-146.
- Stuart RJ, Page RE. 1991. Genetic component to division of labor among workers of a Leptothoracine ant. *Naturwissenschaften.* 78:375-377.
- Tarpy DR. 2003. Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. *Proc R Soc Lond B.* 270:99-103.
- Tarpy DR, Seely TD. 2006. Lower disease infections in honeybee (*Apis mellifera*) colonies headed by polyandrous vs monandrous queens. *Naturwissenschaften.* 93:195-199.
- Thomas ML, Payne-Makrisâ CM, Suarez AV, Tsutsui ND, Holway DA. 2007. Contact between supercolonies elevates aggression in Argentine ants. *Insect Soc.* 54:225-233.
- Van Wilgenburg E, Clémencet J, Tsutsui ND. 2010. Experience influences aggressive behaviour in the Argentine ant. *Biol Lett.* 6:152-155.
- Verbeek MEM, Drent PJ, Wiepkema PR. 1994. Consistent individual differences in early exploratory behaviour in male great tits. *Anim Behav.* 48:1113-1121.
- Wiernasz DC, Perroni CL, Cole BJ. 2004. Polyandry and fitness in the western harvester ant, *Pogonomyrmex occidentalis*. *Mol Ecol.* 13:1601-1606.
- Wilson EO. 1980a. Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*). I. The overall pattern in *Atta sexdens*. *Behav Ecol Sociobiol.* 7:143-156.
- Wilson EO. 1980b. Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*). II. The ergonomic optimization of leaf cutting *Atta sexdens*. *Behav Ecol Sociobiol.* 7:157-165.
- Wilson EO. 1987. Causes of ecological success: the case of the ants. *J Anim Ecol.* 56:1-9.

## Supplementary material

**Table S1.** Results of the original multiple regression (before backward stepwise selection) on the relative colony productivity (annual production in dry weight / number of workers) as the dependant variable. In the model ( $F_{8,30} = 2.51$ ;  $p < 0.033$ ;  $r^2_{\text{adjusted}} = 0.24$ ) were the following explanatory variables included: the number of adult workers, mean colony head width, SD of colony head width, mean colony aggression, SD of colony aggression, mean colony exploration, SD of colony exploration, and nest density (1.5 m<sup>2</sup>). Colony behavior was analyzed by testing 10 ant workers per colony separately in standardized experimental set-ups. A total of 39 colonies were included.

<b>Explanatory variable</b>	<b>Beta</b>	<b>P value</b>
Number of adult workers	-0.039	0.798
Mean colony aggression	-0.371	0.158
SD colony aggression	0.557	<b>0.030</b>
Mean colony exploration	-0.302	0.119
SD colony exploration	0.165	0.392
Nest density (1.5 m <sup>2</sup> )	0.478	<b>0.007</b>
Mean colony head width	-0.096	0.519
SD of colony head width	-0.151	0.333

**Table S2 a.** Results of the original multiple regression (before backward stepwise selection) on the mean colony aggression as the dependant variable. In the model ( $F_{6,32} = 1.84$ ;  $p < 0.124$ ;  $r^2_{\text{adjusted}} = 0.12$ ) were the following explanatory variables included: the number of adult workers, mean colony head width, SD of colony head width, relative colony productivity (dry weight production per worker), mean colony exploration and nest density (1.5 m<sup>2</sup>). A total of 39 colonies were included.

<b>Explanatory variable</b>	<b>Beta</b>	<b>P value</b>
N of adult workers	0.121	0.414
Mean colony head width	-0.137	0.389
SD of colony head width	-0.009	0.958
Relative colony productivity	0.089	0.621
Mean colony exploration	0.213	0.200
Nest density (1.5 m <sup>2</sup> )	0.372	0.053

**Table S2 b.** Results of the best multiple regression model on the mean colony aggression as the dependant variable. The model was selected in a backward stepwise process, first including all explanatory variables and then removing factors until the highest adjusted  $r^2$  value was reached ( $F_{2,36} = 4.97$ ;  $p < 0.013$ ;  $r^2_{\text{adjusted}} = 0.17$ ).

<b>Explanatory variable</b>	<b>Beta</b>	<b>P value</b>
Mean colony exploration	0.217	0.152
Nest density (1.5 m <sup>2</sup> )	0.388	<b>0.013</b>

**Table S3 a.** Results of the original multiple regression (before backward stepwise selection) on the mean colony exploration as the dependant variable. In the model ( $F_{6,32} = 1.07$ ;  $p < 0.41$ ;  $r^2_{\text{adjusted}} = 0.01$ ) were the following explanatory variables included: the number of adult workers, mean colony head width, SD of colony head width, relative colony productivity (dry weight production per worker), mean colony aggression and nest density (1.5 m<sup>2</sup>). Colony behavior was analyzed by testing 10 ant workers per colony separately in standardized experimental set-ups. A total of 39 colonies were included.

<b>Explanatory variable</b>	<b>Beta</b>	<b>P value</b>
N of adult workers	-0.010	0.953
Mean colony head width	-0.019	0.910
SD of colony head width	-0.282	0.106
Relative colony productivity	-0.212	0.262
Mean colony aggression	0.239	0.200
Nest density (1.5 m <sup>2</sup> )	0.170	0.415

**Table S3 b.** Results of the best multiple regression model on the mean colony exploration as the dependant variable. The model was selected in a backward stepwise process, first including all explanatory variables and then removing factors until the highest adjusted  $r^2$  value was reached (Stepwise multiple regression:  $F_{2,36} = 2.65$ ;  $p < 0.09$ ;  $r^2_{\text{adjusted}} = 0.08$ ).

<b>Explanatory variable</b>	<b>Beta</b>	<b>P value</b>
Mean colony aggression	0.260	0.104
SD of colony head width	-0.246	0.123

## Chapter II: Diverse societies are more productive: a lesson from ants

This chapter is based on an original research article accepted in *Proceedings of the Royal Society B* in January 2012.

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Colony of the ant species *Temnothorax longispinosus*.  
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My contributions to this paper are: (i) designing/modifying most of the behavioral experiments, (ii) supervising J.E. Liebmann (bachelor project), (iii) performing the second series of experiments, (iv) statistical analysis of the data, (v) figure development, (vi) writing the first draft and revising it until completion, and (vii) responding to reviewer comments

## Abstract

The fitness consequences of animal personalities (also known as behavioral syndromes) have recently been studied in several solitary species. However, the adaptive significance of collective personalities in social insects and especially of behavioral variation among group members remains largely unexplored. Although intracolony behavioral variation is an important component of division of labor, and as such a key feature for the success of societies, empirical links between behavioral variation and fitness are scarce. We investigated aggression, exploration and brood care behavior in *Temnothorax longispinosus* ant colonies. We focused on two distinct aspects: intercolony variability and its consistency across time and contexts, and intracolony variability and its influence on productivity. Aggressiveness was consistent over four to five months with a new generation of workers emerging in between trial series. Other behaviors were not consistent over time. Exploration of novel environments responded to the sequence of assays: colonies were faster in discovering when workers previously encountered opponents in aggression experiments. Suites of correlated behaviors (e.g. aggression–exploration syndrome) present in the first series did not persist over time. Finally, colonies with more intracolony behavioral variation in brood care and exploration of novel objects were more productive under standardized conditions than colonies with less variation.

**Keywords:** personality, behavioral syndromes, division of labor, fitness, social insects, aggression

## Background

The concept of personality or behavioral syndromes has re-emerged as a way to study the association of consistent behavioral variants with the reproductive success of animals (Dall et al., 2004; Dingemanse and Réale, 2005; Réale et al., 2007). Behavioral syndromes involve: (i) behavioral consistency across time, (ii) consistency of the same behavior across different contexts, or (iii) correlations of different behaviors (Sih et al., 2010). Among the well-studied behavioral traits are aggression, exploration and boldness, which have been found to influence the survival and fitness of individuals in a variety of animal species (Smith and Blumstein, 2008). The effect of a behavioral trait on its owner's fitness is generally context-dependent. While it may be beneficial to be exploratory or bold in a predator-free environment, these behaviors are clearly costly under high predation pressure. The study of suites of correlated behaviors that are consistent across situations or contexts, can help to understand why and how a behavioral type evolved, even if it appears non-adaptive at first sight (Sih et al., 2004b). For example, high aggressiveness in the North American fishing spider was found to decrease the reproductive success of females as it led to pre-copulatory sexual cannibalism, but aggression was beneficial in the context of foraging, resulting in higher feeding rates and increased fecundity (Johnson and Sih, 2005). Consequently, behavioral traits that are frequently associated should be studied together, rather than in isolation (Sih et al., 2004a).

Behavioral syndromes are generally used to describe differences among individuals, i.e. single organisms. In the case of social insects, each colony can be viewed as a superorganism, with a reproductive queen and non-reproductive workers as her extended phenotype (Hölldobler and Wilson, 2009). Indeed, natural selection in social insects does not only act on the individual level, but also on the level of the colony (Korb and Heinze, 2004). This colony level selection can lead to the evolution of colony behavioral variants, for example honeybee colonies were shown to exhibit a collective personality (Wray et al., 2011). Behavioral traits like foraging activity and defensive response were not only part of a behavioral syndrome in honey bees, but correlated with colony productivity. Consequently, personalities can also be studied on the colony level in social insects.

A first goal of this study was to investigate intercolonial behavioral consistency across time (also known as differential consistency; Stamps and Groothuis, 2010) in the ant *Temnothorax longispinosus* in aggressiveness, exploration and brood care. We also explored suites of correlated behaviors, between aggression, exploration and brood care. Furthermore, we tested whether these suites of correlated behaviors were consistent over time and contexts (defined as structural consistency; Stamps and Groothuis, 2010). Some colonies might be very

aggressive and exploratory (proactive), while others could be more shy and cautious (reactive). Similar to solitary animals, proactive colonies are expected to be very competitive and flourish in stable environments, while reactive ones are better in adapting to changes in the environment (Sih et al., 2004a).

Our second goal was to investigate whether intracolony variability in behavior influences colony productivity. Animal groups may not only differ in their average collective personality, e.g. mean aggressiveness, but also in the behavioral variability of their members. While two groups may spend the same total amount of time on brood care, aggressive interactions or foraging, the allocation of tasks among group members could be quite different. In one group, specialists primarily perform a single task, whereas in other groups generalists with a uniform task threshold take over all tasks with the same likelihood. Further, individual task specialization may increase task efficiency. That this is not necessarily the case has been shown recently in *Temnothorax* ants, in which specialists were not more efficient in performing their tasks (Dornhaus, 2008). Nevertheless, colonies with a higher behavioral variation among workers can in theory react faster and more appropriately to changing colony needs. As a result, these colonies should show a more efficient task allocation and higher colony fitness than colonies with less behavioral variability (Myerscough and Oldroyd, 2004).

The ecological success of social insects can be mainly attributed to their division of labor (Hölldobler and Wilson, 1990; Wilson, 1987). Surprisingly, empirical data linking behavioral variation among group members to fitness are still scarce (but see Pruitt and Riechert, 2011). We recently demonstrated a positive relationship between intracolony behavioral variation in aggression and colony productivity in *Temnothorax* ants in the field (Modlmeier and Foitzik, 2011). However, our study also revealed that environmental factors, i.e. habitat quality and population density, can be associated with behavioral variation and could have created the positive correlation between variation in aggression and productivity. High habitat quality could have led to higher productivity and higher ant densities. The latter could have not only increased mean colony aggression but also its variation. Presumably, aggression in ants is generated through a combination of inherited, age-related or environmental factors similar to the honeybees (Alaux et al., 2009).

The present study had three main objectives. First, we asked whether ant colonies differ in behavior even when maintained for several months in the laboratory under the same conditions. We designed three standardized experiments that measured aggression, brood care and exploration behavior of individual ants. In each trial series, 10 workers per colony were



tested, so that we could also study the intracolony behavioral variation in ant colonies. Second, we were interested in the consistency of behavioral variation between colonies over longer time periods (i.e. several months). We tested 10 workers per colony at two points in time, with the development of a new worker generation in between the two testing periods. Finally, we were interested whether there is a relationship between behavioral variation among group members and colony productivity under standardized conditions.

## **Materials and methods**

### **Study system**

*Temnothorax longispinosus* ant colonies were collected in late July 2009 at the Watoga State Park, Pocahontas County, WV, USA. They were transported to our laboratory at the Ludwig Maximilian University Munich, and kept under identical conditions for eight months until the start of the experiment in April 2010. In aggression experiments, ant workers were confronted with a dead conspecific opponent. *Temnothorax longispinosus* colonies from which these opponents were taken were collected at the Huyck Preserve, Albany County, NY, USA, in March–April 2009. Ant colonies were kept in artificial nests in three-chambered plastic boxes (9.5 × 9.5 × 2.7 cm) with a moistened plaster floor in a climate chamber. From July 2009 to the end of September 2009 and again from the end of January 2010, the ant colonies were kept at 20°C : 15°C in a 12 L : 12 D cycle. In-between, ant colonies were kept on a lower temperature cycle to simulate winter conditions (10°C to –5°C). During their active period, ants were fed weekly with honey and pieces of dead crickets. We used 27 monogynous *T. longispinosus* colonies with  $23.11 \pm 7.33$  (mean ± SD) workers.

### **Behavioral experiments**

Ten workers per colony were randomly selected and subsequently used for the brood care, exploration and aggression experiments. Each worker was separately tested and the mean behavior and standard deviation over the 10 workers from a single colony used as a measure of colony behavior and its variation. Brood care was measured first, followed by exploration and finally aggression. Workers from the same colony were always tested in all behaviors on the same day, with about 1 h in between assays. All arenas were wiped with ethanol after each test to remove residual odors and the ethanol was allowed to evaporate for at least 30 s. All experiments were conducted at room temperature (22–25°C).

We conducted two trial series, during each of which 10 workers per colony were tested. The first trial series was conducted between the 5 April and the 2 July 2010 by the second author. The second series, to test for intercolonial behavioral consistency over time was performed between the 8 November and the 5 December 2010 by the first author.

Near the end of the first series of experiments some of the worker pupae began to hatch. We decided not to include freshly emerged callow workers into our experiments as they are known to be mostly inactive during their first days as an adult. Freshly eclosed callow workers are easy to identify because their cuticle is lighter in color. Within two weeks, the cuticle darkens and workers are no longer distinguishable from older workers. Hence, we cannot exclude that some of the workers that were tested close to the end of the trial series eclosed within the last few weeks.

(a) *Brood care behavior*

Each worker was confronted with a worker pupa from its own colony in a small circular arena (diameter 12 mm, height 3 mm) for 5 min. We used pupae instead of larvae for these experiments because the behavior of a larva could influence worker behavior. For example, hungry larvae might beg for food, and variation in begging behavior might thus lead to differences between experiments. Worker behavior was recorded via scan sampling every 20 s. Frequency of pupa grooming was used as a measure of brood care behavior, which is a good measure for overall brood care, because earlier work showed that there were no differences in the tendencies to care for eggs, larvae or pupae among the workers performing brood care (Herbers and Cunningham, 1983). One worker pupa was randomly selected from the workers' colony and re-used for all 10 workers of this colony. We would have preferred to use a new pupa for every worker, but we did not have enough pupae in all colonies. To avoid treating colonies differently, all workers from a single colony were tested with the same pupa.

(b) *Exploration of novel objects and environments*

Single ants were observed in 5 min trials in a multi-chamber set-up consisting of a central circular chamber (diameter 29 mm, height 3 mm) that was connected to eight equally-sized side chambers through eight corridors (length 32 mm, width 7 mm). Each of the eight chambers contained an unfamiliar chemically distinct object: dried pieces of oregano, thyme, rosemary, spruce needles, sage, savory, chamomile and caraway. All objects were exchanged in between tests. As one measure of exploration, we counted how often each worker antennated these objects. This exploration measure is identical to the one used in an earlier

study and can be described as the exploration of novel objects (Modlmeier and Foitzik, 2011). We analyzed a second behavioral aspect of these trials that reflects the exploration of novel environments by the focal ants. This exploration measure was calculated for each ant as the proportion of side chambers that an ant entered ( $n$  of chambers entered/total number of chambers).

### (c) *Aggression*

Aggression was measured by confronting each worker with a freshly defrosted dead non-nest-mate worker from one of 13 opponent colonies from a different population in New York in a small circular arena (diameter 12 mm, height 3 mm). In the aggression experiments, we used dead non-nest-mate conspecifics as opponents, because therefore we can exclude an influence of the opponent's behavior on the outcome of the trial. Moreover, we could show earlier that aggression against dead opponents reflects aggression against live opponents (Modlmeier and Foitzik, 2011). Opponents were killed by freezing. All aggressive interactions (mandible spreading, biting, dragging, carrying and stinging) were recorded every 20 s for 5 min. The frequency of aggressive interactions was calculated by dividing the number of aggressive acts by the number of observations. Opponents were replaced after each encounter.

### **Behavioral consistency on the colony level**

In order to test for consistency in aggressive and exploratory behavior on the colony level, 20 randomly chosen colonies were re-tested after four to five months. We examined only 20 colonies because some of the colonies had died, were too small (contained less than 10 workers) or had lost their queen before the start of the second trial series. Importantly, we were interested in colony personality and therefore killed all tested workers after the first trial series. Consequently, all workers included in the second series were naïve to the behavioral experiments. Moreover, a new generation of workers had emerged in between the two trial series, so that focal workers of the first and second trial series often belonged to two different worker generations. Measuring the colony size before the second trial series allowed us to calculate the percentage of newly hatched workers for every colony. On average, 53.6 per cent of all workers in the second trial had emerged in between the two trial series.

We were unable to re-test brood care behavior during the second trial series in the autumn because during this time of year *T. longispinosus* nests did not contain pupae. Behavioral experiments were identical to the first series with the exception that we evaluated possible order effects of the behavioral assays. Carryover effects from previous assays cannot

only bias results, but also decrease the statistical power and should therefore always be dealt with if possible (Dochtermann, 2010; Logue et al., 2009). To estimate potential order effects, we split the colonies in two treatment groups of 10 colonies each with either aggression tested before exploration (AE) or exploration tested before aggression (EA).

### **Influence of behavioral variation on colony productivity**

As a measure of fitness, we estimated *per capita* productivity (total production divided by colony size), according to a standardized protocol (Modlmeier and Foitzik, 2011). Total biomass production was calculated by counting all male, queen and worker pupae in a colony and subsequently multiplying their number with the average dry weights of the respective castes (Foitzik et al., 2004). As the caste of pre-pupae could not be reliably determined we included them in the calculation as worker pupae. Relative productivity was subsequently calculated for every colony by dividing the total biomass production by the total number of adult workers. The census for the biomass calculation (including the count of colony size) was performed on the 1 April, 4 days before the first series of experiments started. *Temnothorax* ants have a highly synchronized brood production (Headley, 1943), with an emergence of new workers, queens and males in July in the field. However, as our laboratory colonies experienced warmer summer conditions starting already from end of January onwards, brood development was much progressed in April. Most larvae had developed into pupae, which can be easily identified by caste. We only analyzed the association between colony behavior and its variation on productivity during the first trial series.

### **Statistical analyses**

Behavioral data on the individual worker level were not normally distributed, so we used Kruskal–Wallis tests to check for behavioral differences between colonies. By contrast, all data on the colony level were normally distributed (Kolmogorov–Smirnov tests,  $p > 0.2$ ), allowing us to use parametric analyses.

We used factor analysis to investigate suites of correlated behaviors. Principal component or factor analysis are often used to describe behavioral syndromes because they avoid multiple testing (Bell, 2007; Dingemanse et al., 2009). Data for the factor analysis were standardized (Z-score) by subtracting the sample mean from the colony behavior value, the difference was finally divided by the sample standard deviation (Gotelli and Ellison, 2004). All colony behaviors were included in the analysis, but only factors with an eigenvalue greater than one were extracted. Factors were rotated using varimax rotation to facilitate

interpretation. We used Pearson correlation to examine the relationships between individual pairs of behaviors on the colony level. To examine whether behavioral syndromes on the colony level were owing to syndromes on the worker level, we used Spearman rank correlation, because individual data were not normally distributed.

We investigated consistency of colony behavior using analysis of covariance (ANCOVA) with the order of experiments (AE or EA) as a categorical factor, and colony size and original behavior (in the first series) as a continuous predictor. In order to test for consistency of intracolony variation, we repeated this approach for the standard deviation of each behavior (SD aggression, SD exploration (object) and SD exploration (environment)). To analyze potential order effects on the structure of behavioral syndromes, we performed a factor analysis for each treatment separately.

We used multiple regressions to analyze if behaviors or variation in behaviors influence the fitness measure, i.e. relative productivity. In addition, colony size was added as an explanatory variable to the mean colony behaviors (aggression, exploration (object), exploration (environment) and brood care) and SD as a measure of their intracolony variation. As an alternative measure of intracolony variation, we performed the same multiple regression with the intracolony range of behaviors instead of SD.

To further investigate which pattern of behavioral variation is particularly beneficial at the colony level, we calculated moments of distribution, i.e. skewness and kurtosis, for every colony. We performed linear regressions with relative productivity as the dependent and intracolony skewness or kurtosis of the focal behavior as an explanatory variable. These additional analyses were only conducted for those behaviors for which the multiple regression uncovered an association between behavioral variation and productivity. As an alternative to the separate analysis of skewness ( $g_1$ ) and kurtosis ( $g_2$ ), we also calculated degrees ( $\arctan(g_1/g_2)$ ) for all behaviors according to Gilboa & Nonacs (2006) and added degrees into the multiple regression model. Statistical analyses were performed with Statistica 9.1 (StatSoft Inc., Tulsa, Oklahoma, USA).

## Results

### Intercolony behavioral differences

We found strong differences among ant colonies maintained under standardized laboratory conditions for over eight months in brood care, exploration (object), exploration (environment) and aggression (Kruskal–Wallis tests; brood care:  $H_{26,270} = 67.56$ ,  $p < 0.0001$ ;

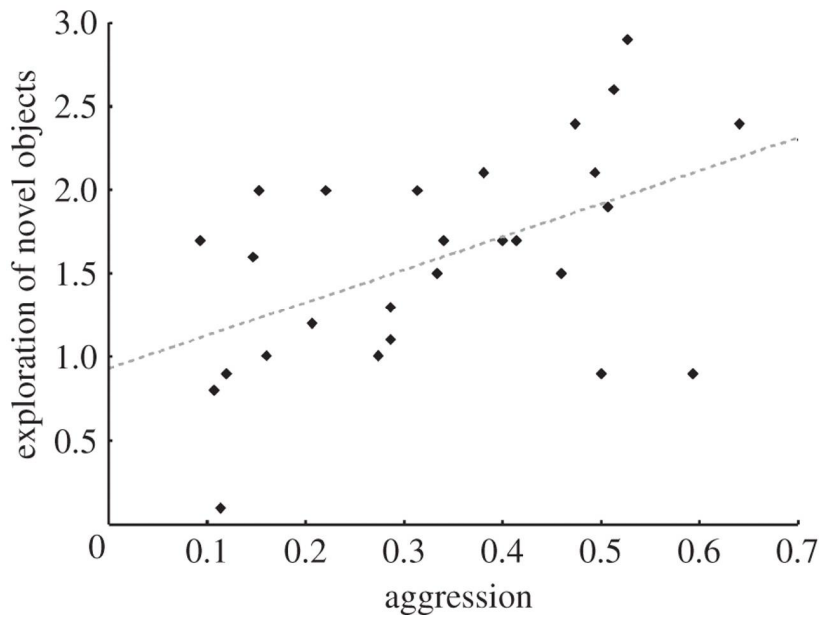
exploration (object):  $H_{26,270} = 51.74$ ,  $p < 0.002$ ; exploration (environment):  $H_{26,270} = 42.54$ ,  $p < 0.03$ ; aggression:  $H_{26,270} = 67.94$ ,  $p < 0.0001$ ).

### Suites of correlated behaviors

Factor analysis revealed a behavioral syndrome on the colony level described by two different factors that, respectively, accounted for 38.5 per cent and 34.5 per cent or a total of 73 per cent of the observed intercolonial behavioral variation in brood care, exploration (object), exploration (environment) and aggression (table 1). The first factor was described by a positive influence of both aggression and exploration (object). The second factor contained heavy loadings from brood care and exploration (environment). Pearson correlation showed a positive relationship between aggression and exploration (object:  $r = 0.507$ ,  $p < 0.01$ ,  $n = 27$ ; figure 1) on the colony level. By contrast, brood care and exploration (environment) were not significantly correlated ( $p = 0.12$ ). No other pairs of behavioral traits were correlated on the colony level ( $p > 0.16$ ). On the individual worker level, we also found the aggression–exploration (object) syndrome (Spearman's  $r = 0.257$ ,  $p < 0.0001$ ,  $n = 270$ ). In addition, brood care and exploration (environment) were positively correlated (Spearman's  $r = 0.144$ ,  $p = 0.02$ ,  $n = 267$ ).

**Table 1.** Factor loadings from factor analysis. Data of all colony behaviors was standardized (Z-score) and only eigenvalues greater than one were extracted. Factors were rotated using varimax rotation. Significant loadings are shown in bold.

Colony behavior	Factor 1	Factor 2
Aggression	<b>0.861</b>	-0.250
Exploration (object)	<b>0.860</b>	0.208
Exploration (environment)	0.158	<b>0.787</b>
Brood care	-0.183	<b>0.811</b>
% variation explained	38.50	34.55

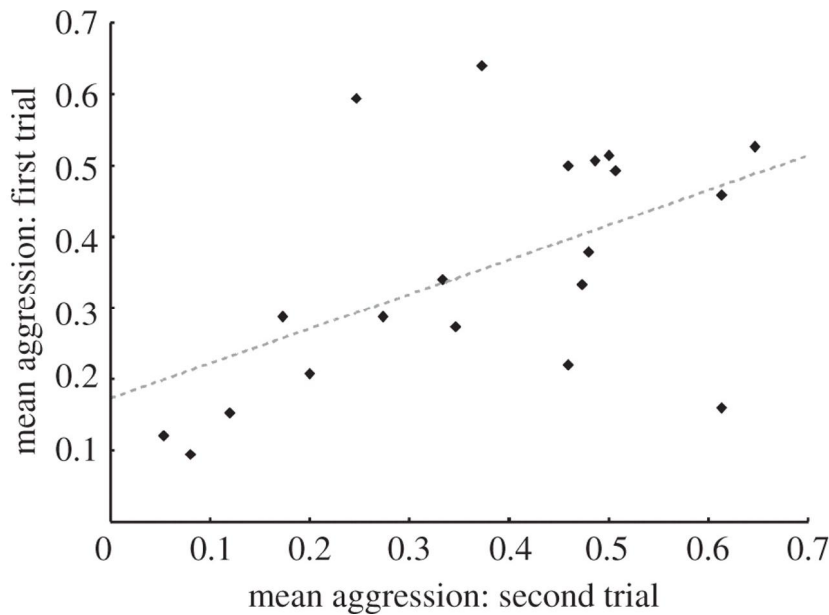


**Figure 1.** Relationship between mean aggression and mean exploration of novel objects of 27 *T. longispinosus* colonies in the first trial series. Ten workers per colony were separately tested to measure mean colony behavior. Pearson correlation gave the following result:  $r = 0.507$ ,  $p < 0.01$ .

### Behavioral consistency on the colony level

Aggressive behavior and intracolony variation in aggressive behavior were consistent on the colony level over a period of at least four to five months (ANCOVA: mean aggression:  $F_{1,16} = 8.55$ ,  $p < 0.01$ ; SD aggression:  $F_{1,16} = 7.33$ ,  $p < 0.02$ ; figure 2). The order of the experiments ( $p > 0.19$ ) and colony size ( $p > 0.63$ ) had no effect on aggression and its variation.

Exploration (object) and its variation could not be explained by any variable ( $p > 0.28$ ), indicating that it is inconsistent on the colony level and not influenced by order effects or colony size. Hence, intracolony variation in both exploratory and aggressive behavior was not correlated to colony size. Exploration (environment) was also inconsistent over time on the colony level ( $p > 0.99$ ), but was influenced by the order of experiments ( $F_{1,16} = 13.47$ ,  $p < 0.01$ ) and by colony size ( $F_{1,16} = 9.68$ ,  $p < 0.01$ ). Colonies, whose workers participated in aggression trials before, showed higher exploration of environment scores. Smaller colonies also had higher exploration scores. Variance in exploration (environment) could not be explained by any variable (all  $p > 0.27$ ).



**Figure 2.** Consistency of aggressive behavior of 20 *T. longispinosus* colonies. Ten workers per colony were separately tested to measure mean colony aggression. The second test was performed four to five months after the first test with the same colony but with different workers. In addition, a new generation of workers emerged in between experiments.

Factor analysis of the second trial series revealed low structural consistency, i.e. correlations between different behaviors from trial series one, were not maintained in the second series. Furthermore, the relationship among behaviors was different in the two treatment groups (table 2). Treatment EA, which is similar to the first trial series, yielded two factors that explained 51.1 per cent and 34.2 per cent, or a total of 85.3 per cent of the observed variation. Factor 1 showed significant negative loadings of exploration (object) and exploration (environment). Factor 2 consisted of one significant negative loading of aggression. Treatment AE generated two factors describing 37.4 per cent and 33.7 per cent, or a total of 71 per cent of the total variation. Aggression and exploration (object) had significant loadings in the first factor, however, in opposite directions. Exploration (environment) was the only significant loading in factor 2. The suggested relationships in treatment EA between exploration (object) and exploration (environment;  $p = 0.11$ ,  $n = 10$ ), and in treatment AE between aggression and exploration (object;  $p = 0.74$ ) were not significant. We also performed correlations on the individual level for these relationships. Individual exploration scores of novel objects and environments were positively associated (Spearman's  $r = 0.224$ ,  $p = 0.025$ ,  $n = 100$ ), whereas aggression and exploration (object) were not correlated on the individual level ( $p > 0.92$ ).



**Table 2.** Factor loadings from factor analysis of the second trial series. Data for aggression, exploration (object) and exploration (environment) behaviors were standardized (z-score) and only eigenvalues greater than one were extracted. Factors were rotated using varimax rotation. Significant loadings are shown in bold.

Colony behavior	Factor 1	Factor 2	Factor 1	Factor 2
	Treatment EA		Treatment AE	
Aggression	0.020	<b>-0.990</b>	<b>-0.741</b>	0.195
Exploration (object)	<b>-0.884</b>	-0.117	<b>0.757</b>	0.178
Exploration (environment)	<b>-0.867</b>	0.178	0.001	<b>-0.970</b>
% variation explained	51.11	34.20	37.39	33.66

### Relative productivity

Colony productivity increased with higher among-worker variability in both brood care ( $\beta = 0.847$ ,  $p < 0.001$ ) and exploration (object) ( $\beta = 0.488$ ,  $p = 0.007$ ; table 3). Interestingly, colony productivity decreased with higher mean values of both brood care ( $\beta = -0.782$ ,  $p < 0.001$ ) and exploration (object) ( $\beta = -0.420$ ,  $p = 0.04$ ). Aggression, exploration (environment) and variation in those behaviors were not associated with colony productivity. Further, colony size did not correlate with productivity ( $p = 0.34$ ). Using an intracolony range of behaviors instead of SD yielded similar results. Both range in brood care behavior ( $\beta = 0.760$ ,  $p = 0.001$ ) and in exploration (object;  $\beta = 0.496$ ,  $p = 0.034$ ) correlated positively with productivity (electronic supplementary material, table S1).

The analysis of skewness and kurtosis revealed that intracolony skewness of brood care behavior was positively related to relative productivity (linear regression:  $r = 0.41$ ,  $p = 0.036$ ). Although intracolony skewness and kurtosis scores were highly correlated (Pearson's  $r = 0.90$ ,  $p < 0.001$ ), intracolony kurtosis values of brood care behavior were not related to productivity ( $p = 0.18$ ). Intracolony skewness and kurtosis values for exploration (object) were not related to productivity ( $p > 0.16$ ). The addition of degrees did not change the results of the original multiple regression model without degrees, and degrees themselves were not correlated with productivity (see the electronic supplementary material, table S2).

**Table 3.** Results of the multiple regression on the relative productivity of 27 laboratory *T. longispinosus* colonies ( $F_{9,17} = 5.62$ ;  $p < 0.001$ ;  $r^2_{\text{adjusted}} = 0.615$ ). Colony behaviors were analyzed by testing 10 workers per colony separately in standardized assays.

<b>Explanatory variable</b>	<b>Beta</b>	<b>P value</b>
Aggression	0.331	0.095
SD aggression	-0.059	0.707
Exploration (object)	<b>-0.420</b>	<b>0.040</b>
SD exploration (object)	<b>0.488</b>	<b>0.007</b>
Exploration (environment)	-0.100	0.479
SD exploration (environment)	-0.087	0.919
Brood care	<b>-0.782</b>	<b>0.001</b>
SD brood care	<b>0.847</b>	<b>0.0002</b>
Colony size	-0.166	0.340

## Discussion

In the present study, we searched for personality differences in aggression, exploration and brood care in *T. longispinosus* ant colonies. Maintenance of ant colonies under common garden conditions over eight months did not reduce or eliminate differences in colony behavior. Ant colonies differed in all analyzed behaviors. We had already shown in an earlier study that colonies tested shortly after the collection in the field differed in aggression and exploration, but could not exclude that these differences were owing to environmental factors (Modlmeier and Foitzik, 2011). Moreover, our results of the second trial series demonstrate that at least in aggression these differences are retained over several months. This indicates that colony personalities are at least partially genetically determined. Alternatively or in addition, developmental processes or social environment could be responsible for the observed consistency. While only aggression remained consistent on the colony level, all behaviors were part of a behavioral syndrome. Most importantly, we found that colonies with higher behavioral variation also showed higher productivity under standardized conditions.

Seventy-three per cent of all behavioral variation in the first trial series could be explained by a behavioral syndrome with two distinct factors. One factor consisted of a positive association between aggression and exploration of novel objects, indicating that aggressive colonies were also more likely to inspect novel objects. This relationship existed on two scales: the colony and the individual level (see also Chapman et al., 2011).

Exploratory colonies were more aggressive because their workers also showed the exploration

(object)–aggression syndrome and not because they contained two separate groups of exploratory and aggressive workers. We speculate that such ‘proactive’ colonies would be bold explorers and at the same time very competitive. This fits the general syndrome termed as the ‘proactive–reactive axis’ that has been found in a number of species (Sih et al., 2004b). The second factor showed a positive relationship between brood care behavior and exploration of novel environments. This is a surprising result, because we did not expect that colonies which spend a lot of time on brood care would also be fast in discovering novel environments. A possible explanation would be that this is an artifact of our experimental design. We randomly chose 10 workers and tested them in situations that they would rarely experience in the field. Workers who rarely leave the nest, e.g. nurses, presumably want to return as fast as possible to their nest to continue with brood care. Thereby, they would discover many chambers without being really interested in exploration.

Aggressiveness and its intracolony variation were consistent on the colony level and not influenced by the exact order of tests. Colonies remained consistent in their aggression for at least four to five months with a new generation of workers emerging in between. Consistency of aggression has already been shown in honeybees and other ant species (Crosland, 1990; Wray et al., 2011). However, we go one step further by examining consistency on the colony level: first, we removed tested individuals after the first experiment. Second, the subsequent experiment was performed after a new generation of workers had emerged. Around 54 per cent of all workers in the second trial series belonged to this new generation. Hence, our results suggest that intercolony variation in aggressiveness was owing to genetic factors, developmental processes and/or social environment. By contrast, neither measure of exploration remained consistent on the colony level. While the positive relationship between aggression and exploration (object) suggests that the latter remains consistent on the individual worker level, its inconsistency on the colony level indicates a lower genetic influence. It seems that exploratory behavior is more influenced by experience. The impact of early experience on ant behavior was shown in an earlier study on the development of foraging behavior (Ravary et al., 2007). Differences in exploratory behavior could also be age-related. Age-related division of labor has been shown in a couple of social insects, e.g. honeybees and some ant species (Calabi et al., 1983; Seeley, 1982). However, in our study species, division of labor is probably not age-related. Sendova-Franks & Franks (1993) found a very weak association between age and task allocation in the closely related *Temnothorax unifasciatus* and further suggested that division of labor is based on workers

‘foraging for work’, i.e. workers look actively for work. Accordingly, even young workers could become foragers, while old workers remain nurses.

So why did we find the positive correlation between exploration (object) and aggression in the first trial series, but not in the second? There are three mechanisms that most probably influence behavioral syndromes: genetics, experience and neuroendocrine effects (Sih et al., 2004b). While the consistency of aggression indicates genetic influence, exploration of objects is probably more strongly affected by experiential factors. In a natural environment, aggressive workers may continue to be curious about new objects, while more peaceful individuals become shyer with every negative experience, i.e. fights with competitors. Therefore, colonies might not have shown a positive relationship between aggression and exploration of objects in the second trial, because they consisted of more workers raised in the laboratory which never lived in a competitive environment. An alternative explanation could be that we by chance tested different castes in the two trial series. Although we randomly picked 10 individuals per colony, we cannot exclude the possibility that we chose many patrollers/guards in the first trial and mainly nurses and foragers in the second trial series. A recent study in *Myrmica* ants showed that the aggression–boldness syndrome (similar to the aggression–exploration of objects syndrome in this study) is only present in the patroller caste and could not be found in the nurses (Chapman et al., 2011).

We further demonstrated that the order of experiments, i.e. if exploration was tested before or after aggression, influenced exploration of novel environments. If workers were confronted with a dead conspecific, they were faster in the discovery of novel environments. After an encounter with a conspecific, workers could be alarmed and try to reach their nest as fast as possible. The different treatments generated two completely different sets of behaviors. The first set (EA treatment) led to a positive relationship between exploration of objects and of environments, while the second (AE treatment) suggested a negative relationship between aggression and exploration (object). This is an important point for future research in the context of behavioral syndromes. One experience alone, in this case an aggressive encounter with a conspecific, cannot only change a single behavior, but moreover create a different relationship among all behaviors. Our result underlines the importance of controlling for order effects when studying behavioral syndromes (Dochtermann, 2010; Logue et al., 2009). Ignoring the sequence of behavioral assays could not only decrease statistical significance, but could also create relationships among behaviors that are owing to order effects. A possible explanation for the observed order effects and the inconsistency of exploration comes from a

study on Argentine ants, showing that these ants can form expectations of their environment from previous experience that will subsequently change how they explore novel habitats (Nonacs and Soriano, 1998). The fact that ants are able to adjust their searching behavior in response to food type (Traniello et al., 1992), microclimatic factors (Azcárate et al., 2007) and group size (Gordon, 1995) also point to a high flexibility of exploratory behavior. Although plasticity in behavior seems to be contradictory to the concept of personality, colonies of harvester ants displayed not only consistent colony-specific differences in foraging activity, but moreover the propensity to adjust to changing food availability (Gordon, 1991; Gordon et al., 2011).

Colony size was not correlated to productivity in the laboratory (this study) and in the field (Modlmeier and Foitzik, 2011). However, our sample size may not be large enough to detect weak effects of colony size. S. Foitzik (2003, unpublished data, based on almost 500 colonies) showed that productivity decreases with colony size (termed as ‘Michener's paradox’; Michener, 1964). Wenzel & Pickering argued that this paradoxical inverse correlation can be explained with the central limit theorem. Accordingly, colony level variation in foraging success decreases with colony size. Small colonies may exhibit the highest productivity, but at the same time suffer more in periods of dearth (Wenzel and Pickering, 1991).

Colony productivity increased with behavioral variation among workers in two behaviors: brood care behavior and exploration of novel objects. This supports our hypothesis that behavioral variation increases colony fitness, presumably through a more efficient task allocation (Myerscough and Oldroyd, 2004). Similar to our earlier study (Modlmeier and Foitzik, 2011), in which we studied productivity in the wild, variation in a behavioral trait increased with productivity. While aggression is probably the most important trait in the context of competition and nest defense, laboratory colonies do not have to compete for resources or defend their nest sites. Hence, laboratory productivity was best explained by two other behaviors, i.e. the among worker variation in exploration of novel objects and brood care. The more variation a colony had in these traits, the more brood they were able to produce. Uniform behavior seems to be detrimental to fitness, especially when colonies reach high mean values in these behaviors. Colonies with many curious and brood caring workers were less productive than colonies with more variation in these behaviors. These results support part of the theoretical model of Myerscough & Oldroyd (2004), i.e. colonies with high behavioral variation may perform better than colonies with uniform behavior. While colonies with a uniform behavior only have an all-or-nothing response to colony needs, colonies with

higher variation show a more effective task allocation, in which an optimal number of workers is allocated to changing task needs. In solitary animals, selection acts on individual phenotypes, e.g. high aggression in Ural owls (Kontiainen et al., 2009) or low boldness in swift foxes (Bremner-Harrison et al., 2004). Social species, however, are under multi-level selection (Korb and Heinze, 2004). In a social group, it may not be optimal to reach a high or low score in a behavior to reach the highest fitness, but instead have the perfect mix of behavioral types (e.g. Modlmeier and Foitzik, 2011; Pruitt and Riechert, 2011). The results of our study suggest that having individuals at the upper and lower bounds (i.e. a larger overall range) of behavior makes the colony more productive. In case of brood care, a more right skewed distribution with many individuals in the lower bounds and a long tail of individuals in the upper bounds of behavior (i.e. specialists or keystone individuals) seems to be the most beneficial pattern of intracolony variation.

If the observed behavioral variation has a genetic basis, then genetic diversity itself could increase productivity in *T. longispinosus*. However, natural selection would probably reduce genetic diversity through directional selection and genetic drift. Nonacs & Kapheim introduced ‘social heterosis’ as a framework to explain why genetic diversity is not continuously reduced through natural selection. They demonstrated mathematically that differences across groups in productivity owing to genetic diversity could counteract both within-group selection and drift (Nonacs and Kapheim, 2007, 2008). The results of our study that productivity increases with intracolony variation in behavior are supportive of social heterosis. Future studies on collective personalities and fitness that include intragroup variation could not only shed light on the evolution of division of labor, but moreover on the adaptive significance of group living in general.

In summary, we were able to show that colonies exhibit behavioral differences in aggression, exploration and brood care despite being held under standardized conditions in the laboratory for eight months. This indicates that the observed differences are not owing to short-term effects of the environment, but innate colony characteristics. All behaviors in the first series of experiments were part of a syndrome that also included an exploration of novel objects–aggression syndrome on two scales (i.e. colony and individual level). Similar to proactive individuals in solitary animals (Sih et al., 2004a), curious colonies were also very aggressive. However, these syndromes did not persist over time. We were further able to demonstrate that colony aggression remains consistent over at least four months with a new generation of workers emerging in between. Therefore, we suggest that aggressiveness in *Temnothorax* ants is at least, in part, genetically and/or developmentally determined.

Exploratory behavior was inconsistent on the colony level indicating a stronger impact of environmental influences, experience or age. One of the observed exploratory behaviors (exploration of novel environments) was strongly influenced by the order in which behaviors were tested, suggesting a plastic response to stimuli such as aggressive encounters. Further, the order of behavioral assays influenced the relationship among all behaviors revealing different suites of correlated behaviors. Our study is therefore a good example for the influence of order effects on behavioral syndromes. Sequence of behavioral experiments should always be included as an explanatory variable if possible (Dochtermann, 2010).

Finally, we were able to show that productivity of ant societies increased with intracolony behavioral variation. In contrast to our earlier study (Modlmeier and Foitzik, 2011), we were able to exclude environmental influences like density on productivity, because we held all colonies under standardized conditions in the laboratory for eight months. This result indicates that colonies with high behavioral variation outperform those with uniform behavior, presumably through a more efficient task allocation. This study therefore suggests that ant societies which show a stronger division of labor are more productive, a fact that has been debated in human societies for centuries (Smith, 1776).

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### **References**

- Alaux C, Sinha S, Hasadsri L, Hunt GJ, Guzmán-Novoa E, DeGrandi-Hoffman G, Uribe-Rubio JL, Southey BR, Rodriguez-Zas S, Robinson GE. 2009. Honey bee aggression supports a link between gene regulation and behavioral evolution. *Proc Natl Acad Sci USA*. 106:15400-15405.
- Azcárate FM, Kovacs E, Peco B. 2007. Microclimatic conditions regulate surface activity in harvester ants *Messor barbatus*. *J Insect Behav*. 20:315-329.
- Bell AM. 2007. Future directions in behavioural syndromes research. *Proc R Soc Lond B*. 274:755-761.
- Bremner-Harrison S, Prodohl PA, Elwood RW. 2004. Behavioural trait assessment as a release criterion: boldness predicts early death in a reintroduction programme of captive-bred swift fox (*Vulpes velox*). *Anim Conserv*. 7:313-320.
- Calabi P, Traniello JFA, Werner MH. 1983. Age polyethism: its occurrence in the ant *Pheidole hortensis*, and some general considerations. *Psyche*. 90:395-412.

- Chapman BB, Thain H, Coughlin J, Hughes WHO. 2011. Behavioural syndromes at multiple scales in *Myrmica* ants. *Anim Behav.* 82:391-397.
- Crosland MWJ. 1990. Variation in ant aggression and kin discrimination ability within and between colonies. *J Insect Behav.* 3:359-379.
- Dall SRX, Houston AI, McNamara JM. 2004. The behavioral ecology of personality: consistent individual differences from an adaptive perspective. *Ecol Lett.* 7:734-739.
- Dingemanse NJ, Dochtermann NA, Wright J. 2009. A method for exploring the structure of behavioural syndromes to allow formal comparison within and between data sets. *Anim Behav.* 79:439-450.
- Dingemanse NJ, Réale D. 2005. Natural selection and animal personality. *Behaviour.* 142:1165-1190.
- Dochtermann NA. 2010. Behavioral syndromes: carryover effects, false discovery rates, and a priori hypotheses. *Behav Ecol.* 21:437-439.
- Dornhaus A. 2008. Specialisation does not predict individual efficiency in an ant. *PLoS Biol.* 6:2368-2375.
- Foitzik S, Backus VL, Trindl A, Herbers JM. 2004. Ecology of *Leptothorax* ants: impact of food, nest sites and social parasites. *Behav Ecol Sociobiol.* 55:484-493.
- Gilboa S, Nonacs P. 2006. Testing models of parental investment strategy and offspring size in ants. *Oecologia.* 146:667-674.
- Gordon DM. 1991. Behavioral flexibility and the foraging ecology of seed-eating ants. *Am Nat.* 138:379-411.
- Gordon DM. 1995. The expandable network of ant exploration. *Animal Behaviour* 50:995-1007.
- Gordon DM, Guetz A, Greene MJ, Holmes S. 2011. Colony variation in the collective regulation of foraging by harvester ants. *Behav Ecol.* 22:429-435.
- Gotelli NJ, Ellison AM. 2004. A primer of ecological statistics. Sunderland, MA: Sinauer.
- Headley AE. 1943. Population studies of two species of ants, *Leptothorax longispinosus* Roger and *Leptothorax curvispinosus* Mayr. *Ann Entomol Soc Am.* 36:743-753.
- Herbers JM, Cunningham M. 1983. Social organization in *Leptothorax longispinosus* Mayr. *Anim Behav.* 31:759-771.
- Hölldobler B, Wilson EO, 1990. *The Ants.* Cambridge, MA.: Harvard Univ. Press.
- Hölldobler B, Wilson EO, 2009. *The superorganism: the beauty, elegance and strangeness of insect societies.* New York, NY: W. W. Norton and Co.
- Johnson JC, Sih A, 2005. Precopulatory sexual cannibalism in fishing spiders (*Dolomedes triton*): a role for behavioral syndromes. *Behav Ecol Sociobiol.* 58:390-396.
- Kontiainen P, Pietäinen H, Huttunen K, Karell P, Kolunen H, Brommer JE. 2009. Aggressive Ural owl mothers recruit more offspring. *Behav Ecol.* 20:789-796.
- Korb J, Heinze J, 2004. Multilevel selection and social evolution of insect societies. *Naturwissenschaften* 91:291-304.
- Logue DM, Mishra S, McCaffrey D, Ball D, Cade WH. 2009. A behavioral syndrome linking courtship behavior toward males and females predicts reproductive success from a single mating in the hissing cockroach, *Gromphadorhina portentosa*. *Behav Ecol.* 20:781-788.
- Michener CD, 1964. Reproductive efficiency in relation to colony size in Hymenopterous Societies. *Insect Soc.* 11:317-342.
- Modlmeier AP, Foitzik S. 2011. Productivity increases with variation in aggression among group members in *Temnothorax* ants. *Behav Ecol.* 22:1026-1032.
- Myerscough MR, Oldroyd BP. 2004. Simulation models of the role of genetic variability in social insect task allocation. *Insect Soc.* 51:146-152.
- Nonacs P, Kapheim KM. 2007. Social heterosis and the maintenance of genetic diversity. *J Evol Biol.* 20:2253-2265.



- Nonacs P, Kapheim KM. 2008. Social heterosis and the maintenance of genetic diversity at the genome level. *J Evol Biol.* 21:631-635.
- Nonacs P, Soriano JL. 1998. Patch sampling behaviour and future foraging expectations in Argentine ants, *Linepithema humile*. *Anim Behav.* 55:519-527.
- Pruitt JN, Riechert SE. 2011. How within-group behavioural variation and task efficiency enhance fitness in a social group. *Proc R Soc B.* 278:1209-1215.
- Ravary F, Lecoutey E, Kaminski G, Châline N, Jaisson P. 2007. Individual experience alone can generate lasting division of labor in ants. *Curr Biol.* 17:1308-1312.
- Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. *Biol Rev Camb Phil Soc.* 82:291-318.
- Seeley TD. 1982. Adaptive significance of the age polyethism schedule in honeybee colonies. *Behav Ecol Sociobiol.* 11:287-293.
- Sendova Franks A, Franks NR, 1993. Task allocation in ant colonies within variable environments (A study of temporal polyethism, experimental). *Bull Math Biol.* 55:75-96.
- Sih A, Bell A, Johnson JC. 2004a. Behavioral syndromes: an ecological and evolutionary overview. *Trends Ecol Evol.* 19:372-378.
- Sih A, Bell A, Johnson JC. 2010. Behavioral Syndromes. In: *Evolutionary Behavioral Ecology* (Westneat DF, Fox CW, eds). New York, NY: Oxford Univ. Press; 516-530.
- Sih A, Bell AM, Johnson JC, Ziemba RE. 2004b. Behavioral syndromes: an integrative overview. *Q Rev Biol.* 79:241-277.
- Smith A. 1776. *An inquiry into the nature and causes of the wealth of nations*. London, UK: W. Strahan and T. Cadell.
- Smith BR, Blumstein DT. 2008. Fitness consequences of personality: a meta-analysis. *Behav Ecol.* 19:448-455.
- Stamps J, Groothuis TGG. 2010. The development of animal personality: relevance, concepts and perspectives. *Biol Rev Camb Phil Soc.* 85:301-325.
- Traniello JFA, Kozol AJ, Fournier MA. 1992. Resource-related spatial patterns of search in the ant *Formica schaufussi*: A field study. *Psyche.* 99:87-83.
- Wenzel JW, Pickering J. 1991. Cooperative foraging, productivity, and the central limit theorem. *Proc Natl Acad Sci USA.* 88:33-38.
- Wilson EO. 1987. Causes of ecological success: the case of the ants. *J An Ecol.* 56:1-9.
- Wray MK, Mattila HR, Seeley TD. 2011. Collective personalities in honeybee colonies are linked to colony fitness. *Anim Behav.* 81:559-568.

## Supplementary material

**Appendix Table S1.** This multiple regression is identical to the model presented in the main manuscript (Table 3) with one difference: as an alternative measurement of intracolony variation, we used intracolony range in behaviors instead of the standard deviation. Results of the multiple regression on the relative productivity of 27 laboratory *T. longispinosus* colonies ( $F_{9,17} = 3.12$ ;  $p < 0.021$ ;  $r^2_{\text{adjusted}} = 0.424$ ). Colony behaviors were analyzed by testing 10 workers per colony separately in standardized assays.

Explanatory variable	Beta	P value
Aggression	0.233	0.368
Range aggression	0.038	0.854
Exploration (object)	-0.316	0.179
Range exploration (object)	<b>0.496</b>	<b>0.034</b>
Exploration (environment)	-0.139	0.418
Range exploration (environment)	0.006	0.971
Brood care	<b>-0.729</b>	<b>0.003</b>
Range brood care	<b>0.760</b>	<b>0.001</b>
Colony size	-0.231	0.235

**Appendix Table S2.** For this multiple regression (which is an extension of table 3 in the main manuscript) we also included degrees of intracolony behavioral variation as a dependent variable. Degrees are used to objectively classify non-normal distributions. Therefore, degrees can be used to investigate what kind of behavioral variation is actually beneficial for the colony. Degrees were calculated as arctan (skewness/kurtosis) for every colony according to Gilboa and Nonacs (2006). Low degree values (10°-60°) represent right skewed or leptokurtic distributions, high values (300°-350°) represent left skewed or leptokurtic distributions. In between these values platykurtic distributions occur. Results of the multiple regression on the relative productivity of 27 laboratory *T. longispinosus* colonies ( $F_{13,13} = 3.67$ ;  $p < 0.013$ ;  $r^2_{\text{adjusted}} = 0.572$ ). Colony behaviors were analyzed by testing 10 workers per colony separately in standardized assays.

<b>Explanatory variable</b>	<b>Beta</b>	<b>P value</b>
Aggression	0.495	0.067
SD aggression	-0.075	0.694
Degree aggression	0.069	0.686
Exploration (object)	<b>-0.557</b>	<b>0.044</b>
SD exploration (object)	<b>0.532</b>	<b>0.008</b>
Degree exploration (object)	0.187	0.341
Exploration (environment)	0.045	0.815
SD exploration (environment)	-0.003	0.987
Degree exploration (environment)	-0.115	0.554
Brood care	<b>-0.817</b>	<b>0.002</b>
SD brood care	<b>0.893</b>	<b>0.0007</b>
Degree brood care	-0.106	0.509
Colony size	-0.119	0.559

### Chapter III: Raiders from the sky: slavemaker founding queens select for aggressive host colonies

This chapter is based on an original research article accepted in *Biology Letters* in June 2012.

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\* These authors contributed equally to this study.



Winged queen of the slavemaking ant *Protomognathus americanus*.  
© Andreas Modlmeier

This study was a joint effort of Tobias Pamminger (TP) and myself. TP wrote the first draft of the manuscript and I revised it until completion. The following contributions were equally shared between TP and myself: (i) conceiving and designing the experiment, (ii) field work, (iii) collection of data and statistical analysis, (iv) figure development, and (v) responding to reviewer comments

## **Abstract**

Reciprocal selection pressures in host–parasite systems drive coevolutionary arms races that lead to advanced adaptations in both opponents. In the interactions between social parasites and their hosts, aggression is one of the major behavioral traits under selection. In a field manipulation, we aimed to disentangle the impact of slavemaking ants and nest density on aggression of *Temnothorax longispinosus* ants. An early slavemaker mating flight provided us with the unique opportunity to study the influence of host aggression and demography on founding decisions and success. We discovered that parasite queens avoided colony foundation in parasitized areas and were able to capture more brood from less aggressive host colonies. Host colony aggression remained consistent over the two-month experiment, but did not respond to our manipulation. However, as one-fifth of all host colonies were successfully invaded by parasite queens, slavemaker nest foundation acts as a strong selection event selecting for high aggression in host colonies.

**Keywords:** parasite, personality, dispersal, aggression, fitness

## Background

Personalities, i.e. consistent inter-individual differences in behavior, affect the fitness of animals in many species, have been shown to be heritable to some degree (Réale et al., 2007) and thus result in potential selection on behavioral traits. In particular, aggressiveness and boldness can increase the reproductive success in competitive environments (Smith and Blumstein, 2008). The fitness consequences of aggression have been demonstrated in multiple species: aggressive females were able to produce more offspring in Ural owls and in red squirrels (Boon et al., 2007; Kontiainen et al., 2009). Aggressive interactions also play an important role in the life of the ant *Temnothorax longispinosus*. In addition to severe intraspecific competition for nest sites in dense populations (Herbers, 1986), this species is the preferred host of the slavemaking ant *Protomognathus americanus* (Herbers and Foitzik, 2002). These obligatory slavemakers are unable to found colonies on their own and invade host colonies where they acquire their first slaves as pupae. In addition, established parasite colonies conduct destructive raids to replenish their slave workforce (Brandt et al., 2005). Contrary to micro-parasites–host associations, slavemakers and their hosts are similar in size and their interactions are mainly behavioral. During slave raids and parasitic queen nest take-overs, hosts and slavemakers behave highly aggressively (Foitzik et al., 2001; Brandt et al. 2005). In both cases, host workers react to the intruding parasite with counter attacks and immediate nest evacuation. Thereby, they can usually rescue some of their brood and the queen.

We previously found that *T. longispinosus* colonies in dense populations are more aggressive (Modlmeier and Foitzik, 2011). This could be either due to natural selection for aggression in crowded habitats or behavioral plasticity as a response to environmental or social conditions. Indeed, a genetic basis of aggression was indicated by behavioral consistency over different worker generations and aggressive colonies could have an advantage under severe intraspecific competition (Modlmeier et al., 2012). On the other hand, host aggression can be to some extent plastic, as aggressiveness towards conspecifics was shown to increase for several days after slavemaker contact (Pamminger et al., 2011). We aimed to identify how natural selection and/or behavioral plasticity shape *T. longispinosus* aggressiveness under natural conditions. We conducted a cross-fostering field experiment in which we manipulated nest density and slavemaker presence. We started the two-month field manipulation in late spring and planned to finish it before the slavemakers' mating flight. However, owing to an exceptionally warm spring, the nuptial flight of the parasite occurred

early that year and about one-fifth of the host colonies were usurped by *P. americanus* queens. This selection event allowed us to study the founding behavior and success of slavemaking queens. In particular, we were able to analyze host nest preference, dispersal and brood take-over success in relation to host aggression and demography under semi-controlled natural conditions. To our knowledge, this is the first time that this critical stage of the parasitic life cycle has been accessible to an experimental investigation.

## Materials and methods

### Study system, field collection and aggression experiments

In May 2010, we collected and censused 16 mature *P. americanus* colonies (each containing a parasite queen and at least two slavemaking workers) and 160 *T. longispinosus* host colonies at the Huyck Preserve, Albany County, NY (42°31'35.3" N, 74°9'30.1" W). Aggression against intruders was determined for all *T. longispinosus* colonies in a standardized set-up by T.P. and A.P.M. (Pamminger et al., 2011; see electronic supplementary material). Colonies of the different treatments did not differ in the nest density of the source area or in aggression (Kruskal–Wallis test:  $p > 0.05$ ). Each colony was allowed to move into an individual artificial nest site (dowel; Herbers, 1986) and placed in field enclosures within two days of collection.

### Field manipulation

During May–June 2010, we constructed eight enclosures in a homogeneous forest area of about 50 × 50 m. Each of the enclosures was composed of four compartments, two larger ones (9 m<sup>2</sup>; 3 × 3 m) and two smaller ones (2.25 m<sup>2</sup>; 1.5 × 1.5 m) that were separated by 40 cm-high aluminum flashing, anchored 10 cm in the ground. The leaf litter and thereby all suitable nest sites were removed from the enclosures to prohibit nest relocation. Thereafter, five *T. longispinosus* colonies in artificial wooden nest sites were placed in each compartment, resulting in nest densities of 2.2 colonies m<sup>-2</sup> in the dense compartments and 0.6 colonies m<sup>-2</sup> in the less dense ones. We placed a slavemaker colony in the center in one of the high- and low-density compartments, creating four different treatments: (i) high density with slavemaker, (ii) high density without slavemaker, (iii) low density with slavemaker, and (iv) low density without slavemaker. All colonies remained in the field for about two months and enclosures were carefully searched for ants in late July. Unless destroyed by raiding or by a parasitic founding queen, we assume that colonies remained in the same artificial nests. We recollected 92 *T. longispinosus* colonies that were censused and subjected to a second standardized aggression test (Pamminger et al., 2011).

## Statistical analyses

To test for consistency in aggressive behavior, we performed an ANCOVA analysis with the aggression after treatment as dependent variable, observer and treatment as categorical predictors, and aggression before treatment as a continuous predictor.

We analyzed how host colony survival was influenced by treatment and host demography using Chi-square-tests and *t*-tests. Further, we investigated whether founding events were related to treatment, host colony size and aggression (see electronic supplementary material).

Take-over success was calculated as the number of brood captured by the slavemaker queen divided by the brood originally present in the colony. We used an ANCOVA analysis with arcsine-transformed percentage of brood captured by the slavemaker queens as the dependent variable and colony aggression as a continuous and observer as a categorical predictor to investigate if more aggressive colonies can evacuate more brood. To control for potential confounding effects (e.g. host colony growth rates), we conducted additional tests (see electronic supplementary material).

## Results

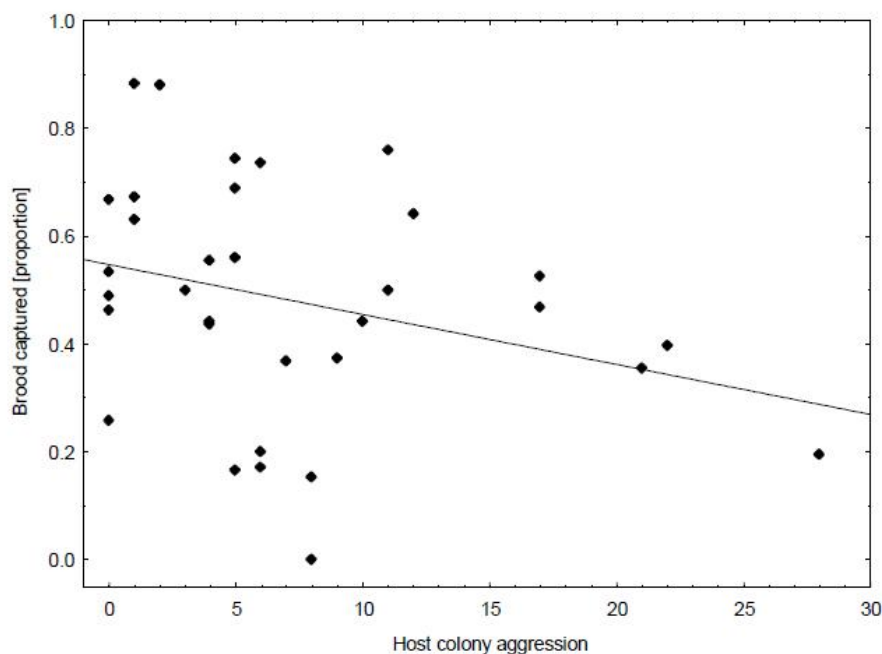
The mating flight of *P. americanus* took place during the manipulation and 21 per cent of our experimental colonies were taken-over by founding *P. americanus* queens. Moreover, a larger fraction of colonies (34%) disappeared from slavemaker treatments than from parasite-free plots (10%;  $\chi^2_1 = 13.2$ ,  $p < 0.0005$ ), suggesting that about a quarter of all colonies in the slavemaker treatments were destroyed during raids. Host colonies that disappeared in slavemaker treatments were smaller than those that survived (*t*-test:  $t = 2.0$ ,  $p < 0.05$ ;  $n_{1,2} = 53, 27$ ), whereas colony size did not affect host nest survivorship in parasite-free experimental plots (*t*-test:  $t = 1.1$ ,  $p = 0.28$ ;  $n_{1,2} = 72, 8$ ).

Treatment, observer and the interactions had no effect on host aggression measured after the experiment (all  $p > 0.05$ ). We found a significant positive correlation between aggression measured before and after the manipulation (ANCOVA: aggression before treatment:  $F_{1,86} = 5.87$ ,  $p < 0.02$ ), demonstrating further that colony identity was largely the same at the end of the experiment.

Moreover, the frequency of successful parasitic colony founding depended on treatment ( $\chi^2_3 = 10.2$ ,  $p < 0.02$ ): while there was no effect of host density ( $\chi^2_1 = 0.3$ ,  $p = 0.56$ ), fewer parasitic founding events occurred in plots in which we released a slavemaker colony



( $\chi^2_1 = 5.8$ ,  $p < 0.02$ ). These analyses were based on the number of host colonies present in the enclosures after the manipulation and thus cannot be explained by the raiding activity of the slavemakers directly. Whether a host colony was taken-over by a parasite queen was unaffected by host colony size ( $t$ -test:  $t = -1.12$ ,  $p = 0.27$ ,  $n_{1,2} = 33, 91$ ) or aggression (Mann Whitney U-test:  $z = 0.39$ ,  $p = 0.70$ ;  $n_{1,2} = 33, 91$ ). However, *P. americanus* queens that invaded more aggressive host colonies obtained a lower percentage of their brood (ANCOVA:  $F_{1,29} = 4.19$ ,  $p < 0.05$ ; figure 1). Observer identity and the interaction did not covary with the percentage of brood captured ( $p > 0.05$ ).



**Figure 1.** The relationship between brood capture rate of founding *Protomognathus americanus* ant queens and host colony aggression of *Temnothorax longispinosus*. Host colony aggression is given as the total number of aggressive interactions. Presented are 33 founding events from a field experiment.

## Discussion

The external conditions for *T. longispinosus* colonies at our study site are favorable, leading to high nest densities and colony productivity. However, these ant colonies suffer from severe competition for nest sites and food (Herbers, 1986; Foitzik et al., 2004, 2009) and are regularly attacked by social parasites trying to steal their brood (Foitzik and Herbers, 2001). In such an environment dominated by antagonistic interactions, aggression should be favored. Indeed, we found that more aggressive host colonies were able to rescue a higher fraction of their brood from invading slavemaker queens. Aggression did not play a role in parasite

aversion (take-over rate was not influenced by host colony aggression); instead, more aggressive host colonies were able to escape with a higher proportion of brood, which should translate into a fitness benefit. The selection pressure through parasitic nest foundations was found to be high, as 20 per cent of the host colonies were successfully usurped by slavemaking queens within one season. In addition, we can demonstrate that about a quarter of the host colonies were destroyed during raids and smaller host nests were destroyed more often. Hence, the combination of parasitic founding and raiding events selects for larger and more aggressive host colonies. From the parasite perspective, host aggression strongly influences the future of the slavemaking nest, as parasite queens, which obtained only a low fraction of the host brood will start their colony with only few slaves.

Our density and parasite treatments did not influence host colony aggression, but the latter was correlated over two months in the field. This is in accordance with earlier work showing consistency in colony aggression over different worker generations (Modlmeier et al., 2012). Albeit behavioral experiments showed that host colonies respond to slavemaker contact with an induced short-term increase in aggression against conspecifics (Pamminger et al., 2011), the presence of a slavemaking colony within a plot did not result in higher aggression of host colonies. Possibly, the encounter frequency of host colonies with slavemakers in parasitized plots is too low to lead to a consistent increase in aggression. Nevertheless, slavemaker queens invaded host colonies less often in experimentally parasitized plots. This can be explained by host nests in parasitized plots exhibiting a better nest defense either because parasite encounters induced aggressive responses specific to slavemakers or because slave raids selected for better-defended host nests. Alternatively, slavemaking queens might have been able to detect slavemaking colonies by their odor and actively avoid competition with already established slavemaking nests. Indeed, slavemaking queens of the related species *Harpagoxenus sublaevis* do not return to their home locale, but actively search for new host colonies (Buschinger, 1974).

Our experiment allowed us to analyze how a personality trait and demography influence the ability of host colonies to defend themselves against parasitic invasions. Parasite queens obtained less brood from aggressive host colonies, and thereby selected for host aggression. Furthermore, slavemaking queens actively avoided invading host nests in parasitized areas and/or the conditions prohibited successful invasion that should influence parasite distribution within the host population. This study adds another dimension to the growing knowledge of the influence of parasites on animal personalities (Barber and Dingemans, 2010).

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## References

- Barber I, Dingemanse NJ. 2010. Parasitism and the evolutionary ecology of animal personality. *Philos Trans R Soc B*. 365:4077-4088.
- Boon AK, Réale D, Boutin S. 2007. The interaction between personality, offspring fitness and food abundance in North American red squirrels. *Ecol Lett*. 10:1094–1104.
- Brandt M, Foitzik S, Fischer-Blass B, Heinze J. 2005. The coevolutionary dynamics of obligate ant social parasite systems--between prudence and antagonism. *Biol Rev Camb Phil Soc*. 80:251-267.
- Buschinger A. 1974. Experimente und Beobachtungen zur Gründung und Entwicklung neuer Sozietäten der sklavenhaltenden Ameise *Harpagoxenus sublaevis* (Nyl.). *Insect Soc*. 21:381-406.
- Foitzik S, Achenbach A, Brandt M. 2009. Locally adapted social parasite affects density, social structure, and life history of its ant hosts. *Ecology*. 90:1195-1206.
- Foitzik S, Backus VL, Trindl A, Herbers JM. 2004. Ecology of *Leptothorax* ants: impact of food, nest sites and social parasites. *Behav Ecol Sociobiol*. 55:484-493.
- Foitzik S, Deheer CJ, Hunjan DN, Herbers JM. 2001. Coevolution in host-parasite systems: behavioural strategies of slave-making ants and their hosts. *Proc Roy Soc B*. 268:1139-1146.
- Foitzik S, Herbers JM. 2001. Colony structure of a slavemaking ant. II. Frequency of slave raids and impact on the host population. *Evolution*. 55:316-323.
- Herbers JM. 1986 Nest site limitation and facultative polygyny in the ant *Leptothorax longispinosus*. *Behav Ecol Sociobiol*. 19:115-122.
- Herbers JM, Foitzik S. 2002. The ecology of slavemaking ants and their hosts in north temperate forests. *Ecology*. 83:148-163.
- Kontiainen P, Pietäinen H, Huttunen K, Karell P, Kolunen H, Brommer JE. 2009. Aggressive Ural owl mothers recruit more offspring. *Behav Ecol*. 20:789–796.
- Modlmeier AP, Foitzik S. 2011. Productivity increases with variation in aggression among group members in *Temnothorax* ants. *Behav Ecol*. 22:1026-1032.
- Modlmeier AP, Liebmann JE, Foitzik S. 2012. Diverse societies are more productive: a lesson from ants. *Proc Roy Soc B*. 279:2142-2150.
- Pamminger T, Scharf I, Pennings PS, Foitzik S. 2011. Increased host aggression as an induced defense against slave-making ants. *Behav Ecol*. 22:255-260.
- Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. *Biol Rev Camb Phil Soc*. 82:291-318.
- Smith BR, Blumstein DT. 2008. Fitness consequences of personality: a meta-analysis. *Behav Ecol*. 19:448-455.

## Supplementary material

### Methods

#### *Aggression tests*

We measured colony aggression by introducing a frozen conspecific non-nestmate. The usage of a dead adversary removed any effects of the opponents' behavior and was shown to reflect host colony aggression against live conspecifics (Modlmeier and Foitzik 2011). All interactions of the host colony to the dead opponent were recorded by scan sampling every 20 seconds in the first minute and every 30 seconds for the following 4 minutes resulting in a total of 11 observations. We recorded six behavioral categories: antennation (as a non-aggressive interaction) and mandible spreading (threat display), biting, holding, dragging and stinging as aggressive interactions. An aggression index was calculated as the sum of all aggressive acts within 5 min.

#### *Host colony survival*

We analyzed whether host colonies survived more often in parasite-free plots using a  $\chi^2$ -test. Furthermore, we tested separately for parasitized and unparasitized plots whether surviving colonies were larger or smaller than those which perished by using a t-test based on log-transformed colony size (N of workers).

#### *Slavemaker founding event*

We investigated the decision making or invasion success of *P. americanus* founding queens by analyzing how the following parameters influence frequency of host colony invasion: slavemaker presence (frequency of founding events within slavemaker treatments), host density and colony size. We performed  $\chi^2$ -tests to examine whether founding queens preferentially invaded host colonies of a certain treatment. First, we tested for differences in the frequency of successful parasitic colony founding between all four treatments in a 2x4 matrix. Then, we compared founding preferences for density and slavemaker presence separately using 2x2 matrices. The effect of colony size and host aggression on whether or not a host colony was invaded was evaluated by a t-test using log-transformed colony size (N of workers) and a Mann-Whitney U test, respectively. These tests included only host colonies that were still present at the end of the manipulation.

### *Potential relationship between host aggression and growth rate*

We correlated aggressiveness with growth rates in *T. longispinosus* colonies that were not overtaken by *P. americanus*. Growth rate was calculated as the number of brood/callows at the end of the experiment divided by the number of brood before the experiment. We performed an ANCOVA that was similar to the one used to calculate take-over success. Accordingly, growth rate was the dependent variable, colony aggression (at the start of the experiment; as in the takeover success analysis) the continuous and observer the categorical predictor. The results revealed that growth rate was not related to colony aggression (ANCOVA:  $F_{1,85} = 0.05$ ,  $p = 0.83$ ). Hence, we were able to exclude the possibility that the observed relationship between host aggressiveness and brood capture rate of the slavemaker queen is due to a correlation between colony growth rates and aggression.

### *Density treatment after the experiment*

Despite the loss of *T. longispinosus* colonies in all treatments, and a consequent drop of host density in all treatments, we recovered the original ratio of 4:1 in host density between high and low density treatments. This indicated that the density differences between treatments were present during the entire experiment.

## Chapter IV: Age and ovarian development influence worker personality in the ant *Leptothorax acervorum*

This chapter is based on the following manuscript:

Kühbandner S, **Modlmeier AP**, Foitzik S. Age and ovarian development influence worker personality in the ant *Leptothorax acervorum*. To be submitted



Workers of the ant species *Leptothorax acervorum*.  
© Andreas Modlmeier

My contributions to this manuscript are: (i) participation in the experimental design, (ii) performing the statistical analysis, (iii) writing most of the results/discussion sections and revising all other sections, (iv) figure development, and (v) journal correspondence

## Abstract

Behavioral syndromes (i.e. personalities) are found in many animals, in which individuals behave consistently through time and across contexts. In social insects, workers of different morphological castes and age are known to act differently. Yet, it is unclear how body size and ovarian development influence ‘behavioral syndromes’ in similar aged ant workers of monomorphic species. We investigated individual behavior in *Leptothorax acervorum* ant workers at two time points during the first three months of their life and in two different settings. We observed worker behavior in the nest and in standardized aggression, exploration and brood care experiments and found behavioral repeatability in foraging and exploration. Further, workers acted consistently across settings: active workers in the nest also explored more in standardized experiments. Moreover, ovarian development influenced worker behavior: workers with well-developed ovaries foraged less, but were more aggressive. In accordance with the typical age-polyethism of social insects, workers became more active, foraged and explored more as they grew older. Hence, division of labor in these ants arises not only from age-related changes in behavior, but also from consistent behavioral differences of workers of the same age class, which are in turn affected by their ovarian activity.

**Keywords:** personality, behavioral syndrome, division of labor, ovarian development, foraging, social insects, *Leptothorax*

## Background

Behavioral syndromes (i.e. personalities) in social insects have drawn much attention during the last few years: studies focused either on collective personalities of whole colonies (e.g. Gordon et al. 2011; Wray et al. 2011; Pinter-Wollman et al. 2012; Scharf et al. 2012) or on variation in personalities between single individuals (e.g. Muller et al. 2010; Chapman et al. 2011; Modlmeier et al. 2012). Although the concept of personality has only recently been used to describe consistent behavioral differences in social insects, individuals in insect societies have long been known to consistently differ in their behavior and in the tasks they undertake (Hölldobler and Wilson 1990). A characteristic trait of hymenopteran societies is reproductive division of labor: queens are morphologically and behaviorally specialized on reproduction, while workers are either entirely sterile or else show a much lower fertility (Robinson 1992; Glennis and Cahan 1999). Moreover, in most social insects, and especially in ants, workers show a non-reproductive division of labor. Some workers focus on offspring care, whereas others forage or defend the nest. Task specialization is thought to increase worker efficiency, albeit recent findings indicate that this is not always the case (Dornhaus 2008).

But how are the different tasks distributed among the work force? There are two common patterns of division of labor: temporal polyethism (i.e. age-polyethism) and morphological polyethism (for reviews on division of labor see: Gordon 1996; Beshers and Fewell 2001). Tasks typically change with age: young individuals perform tasks inside the nest, like brood care and nest cleaning, before they switch to foraging and other risky activities outside the nest (Seeley 1982; Calabi et al. 1983). Morphological polyethism describes task allocation according to body size. Some ant species even exhibit morphologically distinct worker castes, e.g. minors, majors, or soldiers, which strongly differ in their behavior and the tasks they perform (Wilson 1980). Larger workers (majors) are usually engaged in external defense tasks, while smaller ones (minors) show more care and foraging behavior (Waser 1998; Fournier et al. 2007). Other important influences on behavior appear to be social interactions, experience, and genetic or environmental factors (Robinson 1992; Gordon 1996; Ravary et al. 2007). In honey bees, the occurrence of different worker patriline within a single hive due to multiple mating of the queen increases variability in behavior and body size of workers (Frumhoff and Baker 1988; Robinson and Page 1988; Chapman et al. 2007; Oldroyd and Fewell 2007). Similarly, workers of different patriline of the leaf-cutting ant *Acromyrmex echinator* are specialized on behavioral tasks like waste-management or foraging (Waddington et al. 2010). Yet in *Cataglyphis* ants behavioral caste was uncorrelated to



patriline and mainly depended on body size (Fournier et al. 2007). Last but not least, physiology can influence task allocation: in honeybees, foraging behavior is regulated by their reproductive physiology (Amdam et al. 2004; Siegel et al. 2012). Further, ovarian development was connected to division of labor among workers of different ant species (e.g. Bourke 1988; Fénelon et al. 1996; Pohl et al. 2011). In harvester ants, high levels of ovarian-produced ecdysteroids resulted in a specialization on inside tasks, demonstrating the importance of endocrine physiology for the division of labor in workers (Dolezal et al. 2012).

Here, we study the influence of body size and ovarian development on behavior and task allocation among similar-aged workers in the monomorphic ant species, *Leptothorax acervorum*. This ant has small colonies with one to several queens and on average less than 100 workers. At two points during their early life, we analyzed whether workers of the same age differ consistently in their behavior and if yes, which parameters co-vary with the different behavioral types. To search for relationships between behaviors that are known to be part of behavioral syndromes in other species (Bell 2007), we observed workers both in the nest setting and in standardized aggression, exploration and brood care experiments. Finally, we measured body size and ovarian development to study the associations of these morphological traits to worker behavior. Based on earlier work on other social insect species we derived the following hypotheses and predictions:

- 1) Age-polyethism also occurs in *L. acervorum* and we predict a shift in worker behavior from inactivity and social behaviors to more outside work during the first three months of the workers life.
- 2) Workers should not only show behavioral consistency over time, but also suites of correlated traits.
- 3) We expect that morphology and physiology influence worker behavior. In particular, we predict based on earlier studies on ants (e.g. Bourke 1988; Fénelon et al. 1996; Pohl et al. 2011; Dolezal et al. 2012), that workers with more developed ovaries perform tasks inside the nest, while workers with small degenerated ovaries forage. If morphology is important for worker behavior, we would expect that larger workers take over external tasks like foraging and nest defense, while smaller ones perform more brood care.

## Materials and methods

### Ant colonies

The *Leptothorax acervorum* colonies were collected in summer 2008 in the Reichswald near Nuremberg, Germany. We kept the colonies in our laboratory in three chamber nests described elsewhere (Buschinger 1974; Heinze and Ortius 1991). They were fed three times per week with honey and crickets. Colonies were kept in the laboratory under these standardized conditions for six months before we started the experiments.

### Markings with wire

Workers were individually marked with three differently colored wires with a diameter from 0.02 to 0.03  $\mu\text{m}$ . Small pieces of wire were looped around the ant at three different parts of the body: head and thorax, petiole and postpetiole, or postpetiole and gaster (Mirenda and Vinson 1979). The ends of the wires were shortened, so they did not restrict the movement of the worker. With this method 110 randomly chosen workers could be marked in nine colonies, one week after eclosion from the pupae. It was necessary to wait a week, because attempts to mark ants earlier were unsuccessful as the cuticle of freshly eclosed ants was too soft and nestmates showed aggressive behavior towards them. One week old ants ('callows') could be still reliably recognized by their lighter cuticle.

### Scan Samplings in the nest setting

In order to record the behavior of all marked workers in the nest setting, we performed two series of scan samplings. Observations for the first series started on average  $16.8 \pm 0.6$  days (mean  $\pm$  SE) after eclosion and lasted for  $27.0 \pm 1.4$  days (n of observations per ant:  $12.0 \pm 0.4$ ). The second series started  $92.6 \pm 0.9$  days after eclosion and lasted for 20 days (n of observations per ant:  $34.0 \pm 0.4$ ). If an individual could not be detected during a scan, this was treated as a missing value and was not included in the total number of observations. The observed behavior was classified using the following categories: foraging (walking outside the nest), social care, and resting. Social care was defined as feeding and cleaning of nestmates, and all behaviors related to brood care (carrying, cleaning and feeding of brood). Resting was defined as being inactive, i.e. not moving or interacting with others. When more than one scan was made on a single day (range: 1-3 scans per day), observations were carried out at intervals of at least two hours. The observed behavior was categorized and the relative percentage of each behavior was calculated.

### **Individual behavior in standardized experiments**

Experiments were carried out in two series  $38.5 \pm 0.6$  and  $67.8 \pm 0.2$  days after eclosion and consisted of individual aggression, brood care and exploration experiments. Both the aggression and the brood care experiments were conducted within a small test arena with a diameter of 1.5 cm and a height of 0.3 cm. Individual behavior was recorded every 30 seconds for 10 minutes. First, aggression to non-nestmates was tested using freshly frozen and defrosted workers from another, so-called opponent colony. We used dead ants to exclude potential effects of the opponents' behavior. Further, it has been shown that aggression to dead workers is positively correlated to aggression to live opponents (Modlmeier and Foitzik 2011). Aggressive interactions included mandible spreading (threat display), biting, dragging, carrying and stinging. Second, in the brood care experiment a worker was tested with a worker pupa from the same nest. Brood care behavior included cleaning, carrying and guarding of the pupa (i.e. prolonged contact). Afterwards, the relative percentages (number of times behavior x was observed / total number of observations) for each behavioral category were calculated. Third, for the exploration experiment a special test arena was constructed with one central arena (diameter 29 mm and height 3 mm) surrounded by eight side chambers which were connected with the central arena via corridors (length 32mm and width 7 mm). To investigate exploration of novel objects, small pieces of trees and spices (spruce needle, oregano, caraway, thyme, sage, chamomile, common European oak wood, one chamber was empty) were put into the side chambers (similar to Modlmeier and Foitzik 2011). After the worker was released in the central chamber, we recorded the number of antennation events with all objects (exploration of novel objects) and calculated the percentage of chambers entered (exploration of novel environment) within 10 minutes.

### **Measurements of head width and ovary development**

To investigate the influence of body size on behavior the worker, head width was measured to the nearest 0.01 mm using a stereomicroscope (Zeiss Stemi 2000 C) with an object micrometer and 128-fold magnifications. The influence of reproductive state of the workers was investigated through dissection of the gaster and measurement of the ovaries. This was done under a stereomicroscope with an object-micrometer and 41-fold magnification after the end of the second trial series. The ovaries of *L. acervorum* workers consist of two ovarioles and we measured the length of both and calculated a mean as a measure of fertility status (Konrad et al. 2012). Ants were frozen and killed before ovary dissection, so that it was impossible to measure ovary length at the end of the first trial series. Hence, we analyzed the

influence of ovary length after the second trial series on behaviors of the first and second trial series.

## **Statistics**

### *Changes in worker behavior with age*

Comparing the individual behavior of each ant in both trial series enabled us to investigate changes in worker behavior that are due to age and/or experience. We decided to use non-parametric Wilcoxon-signed-rank test to investigate age-specific differences in behavior between the two trial series, because only exploration of novel environments and resting in trial series one were normally distributed (Kolmogorov–Smirnov test:  $P > 0.10$ ), while all other behaviors were not (K-S test:  $P < 0.05$ ).

### *Repeatability of behavior*

To calculate the repeatability of individual behavior between the two trial series, we used a generalized linear mixed-effects model (GLMM) with logit or log link following the guidelines outlined in Nakagawa and Schielzeth (2010), and the R package ‘rptR’ (<http://r-forge.r-project.org/projects/rptr>) for the free programming language R (R Development Core Team 2009). As the behavioral data consisted of count (exploration of novel objects) and proportion data (all other behaviors), we calculated repeatability with multiplicative overdispersion models fitted by PQL with 1000 permutations using the R functions ‘rpt.binomGLMM.multi’ (overdispersed binomial GLMM with logit link) for proportion data and ‘rpt.poisGLMM.multi’ (overdispersed Poisson GLMM with log link) for count data. We calculated both link scale and original scale repeatability. While link scale repeatability estimates the underlying (latent) repeatability of behavior, original scale repeatability measures the reproducibility of actual measurements (Nakagawa and Schielzeth 2010).

### *Suites of correlated behavioral traits*

We investigated suites of correlated behaviors in similar-aged workers and also if these suites are consistent across time (defined as structural consistency; Stamps and Groothuis 2010) by performing a principal component analysis (PCA) each for the first and the second trial series. PCAs are frequently used to study behavioral syndromes because they circumvent multiple testing (Dingemanse et al. 2010). Prior to the PCA, we performed a z-score transformation for all individual scores for each colony, i.e. we subtracted the mean colony value from the individual scores and divided by the colony standard deviation (Gotelli and Ellison 2004). We

included all z-score transformed behaviors of the standardized experiments (aggression, brood care and both exploration measures) and behaviors in the nest setting (foraging, activity and social care), but only extracted factors with an eigenvalue greater than one. To facilitate interpretation, components were rotated using varimax rotation (Gotelli and Ellison 2004). According to Comrey and Lee (1992), only loadings larger than 0.32 are discussed.

### *Influence of head width and ovary length*

To study how morphology and physiology are related to suites of correlated behavioral traits, we correlated the PCs of the first and second trial series against the z-score transformed head width and ovary length with a Pearson correlation. Unless otherwise stated, statistical analyses were performed with Statistica 9.1 (StatSoft Inc., Tulsa, Oklahoma, USA).

## **Results**

### **Changes in worker behavior with age**

In the nest setting, workers rested more and foraged less during the first trial series compared to the second series (i.e. when they were younger) (Wilcoxon signed-ranks test; resting:  $T = 325$ ,  $N = 66$ ,  $P < 0.0001$ ; foraging:  $T = 38$ ,  $N = 20$ ,  $P = 0.012$ ). In the standardized experiments, workers explored fewer novel environments ( $T = 451$ ,  $N = 53$ ,  $P = 0.019$ ) during the first trial series. All other behaviors did not change with age ( $P > 0.52$ ).

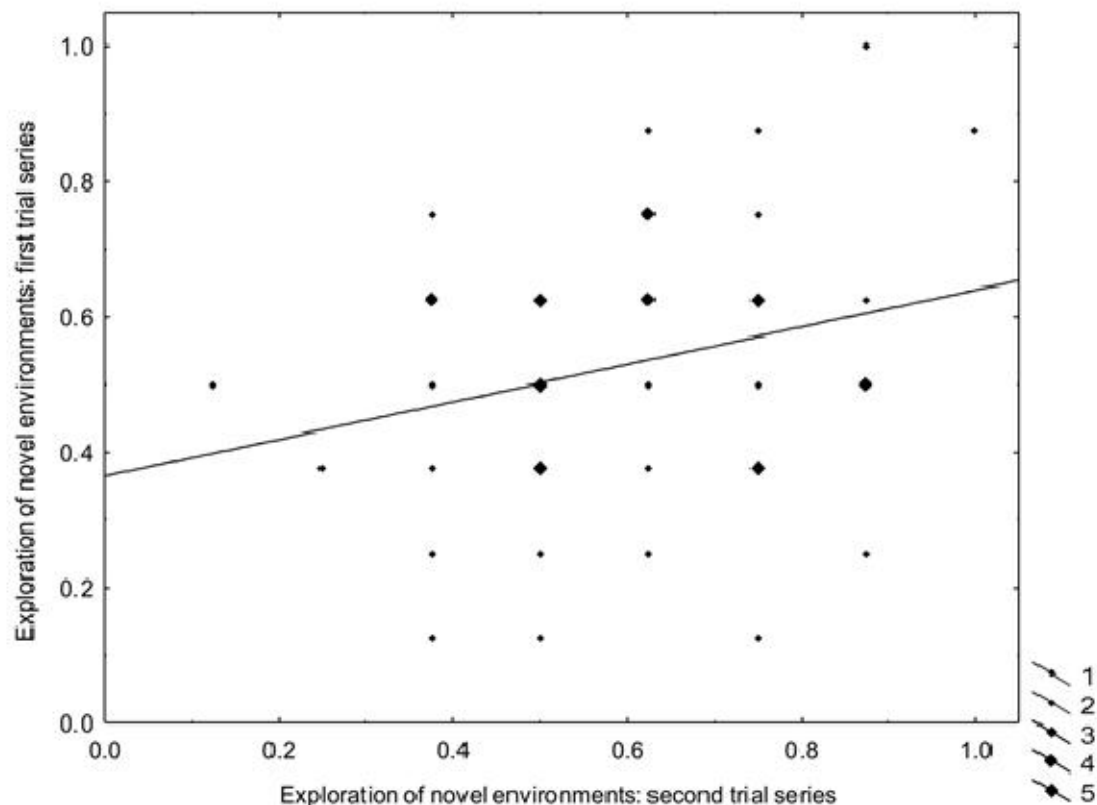
### **Repeatability of behavior**

In the nest setting, foraging was highly repeatable on the latent scale (GLMM:  $R = 0.71$ ,  $P = 0.01$ ; Table 1), while the repeatability of actual measurements (i.e. on the original scale) was lower ( $R = 0.04$ ,  $P = 0.02$ ). This is in line with the results of the Wilcoxon signed-rank test showing an increase in foraging activity with age. Resting (i.e. how active an individual was) possessed low original and link scale repeatability (both scales:  $R = 0.02$ ,  $P = 0.02$ ). Social care was not repeatable ( $P = 0.65$ ).

In the standardized experiments, only exploration behavior (environment) was repeatable ( $R_{\text{orig/link}} = 0.03/0.04$ ,  $P = 0.03$ ; Figure 1). In addition, exploration (object) showed a weak trend ( $R = 0.29$ ;  $P = 0.10$ ) Aggression and brood care behavior were not repeatable ( $P > 0.12$ ).

**Table 1.** Summary of behavioral repeatability estimates of 66 *L. acervorum* workers displaying the results of GLMMs using multiplicative overdispersion models fitted to PQL. Individual workers were tested in two trial series in standardized experiments (aggression, brood care and exploration) and in the nest setting (foraging, resting, social care). Presented are link (underlying) scale and original scale (of actual measurements) repeatabilities. Significant P-values are given in bold.

Behavior	Link scale			Original scale		
	R	95% CI	P	R	95% CI	P
Aggression	0.14	0.08-0.35	0.13	0.02	0.01-0.06	0.13
Brood Care	0.00	0.00-0.03	0.75	0.00	0.00-0.02	0.75
Exploration (object)	0.29	0.10-0.53	0.10	0.29	0.09-0.59	0.10
Exploration (environment)	0.04	0.00-0.09	<b>0.03</b>	0.03	0.00-0.08	<b>0.03</b>
Foraging	0.71	0.00-0.92	<b>0.01</b>	0.04	0.00-0.13	<b>0.02</b>
Resting	0.02	0.00-0.05	<b>0.02</b>	0.02	0.00-0.04	<b>0.02</b>
Social Care	0.00	0.00-0.21	0.65	0.00	0.00-0.02	0.65



**Figure 1.** Behavioral repeatability: exploration of novel environments of 66 same-aged *L. acervorum* workers measured about one month and two months after eclosion. Behavior was measured separately for every worker in standardized experiments. Given is a frequency scatterplot with numbers representing the frequency of overlapping points.

### Suites of correlated behavioral traits

Principal component analysis of the first series of experiments (~ 1 month after worker eclosion) gave four PCs with an eigenvalue larger than one explaining 72.1 % of the total variation in behavior (Table 2). The first PC revealed that workers performing more social care (nest), rest less (i.e. are more active in the nest) and are less aggressive (trial). The second PC suggested that aggressive workers perform less brood care in the standardized tests. Further, both exploratory behaviors, i.e. exploration of novel objects and of novel environments, are tightly linked in an overall exploration syndrome in PC 3. The fourth PC showed that individuals who spent more time on foraging spent less time on resting in the nest setting.

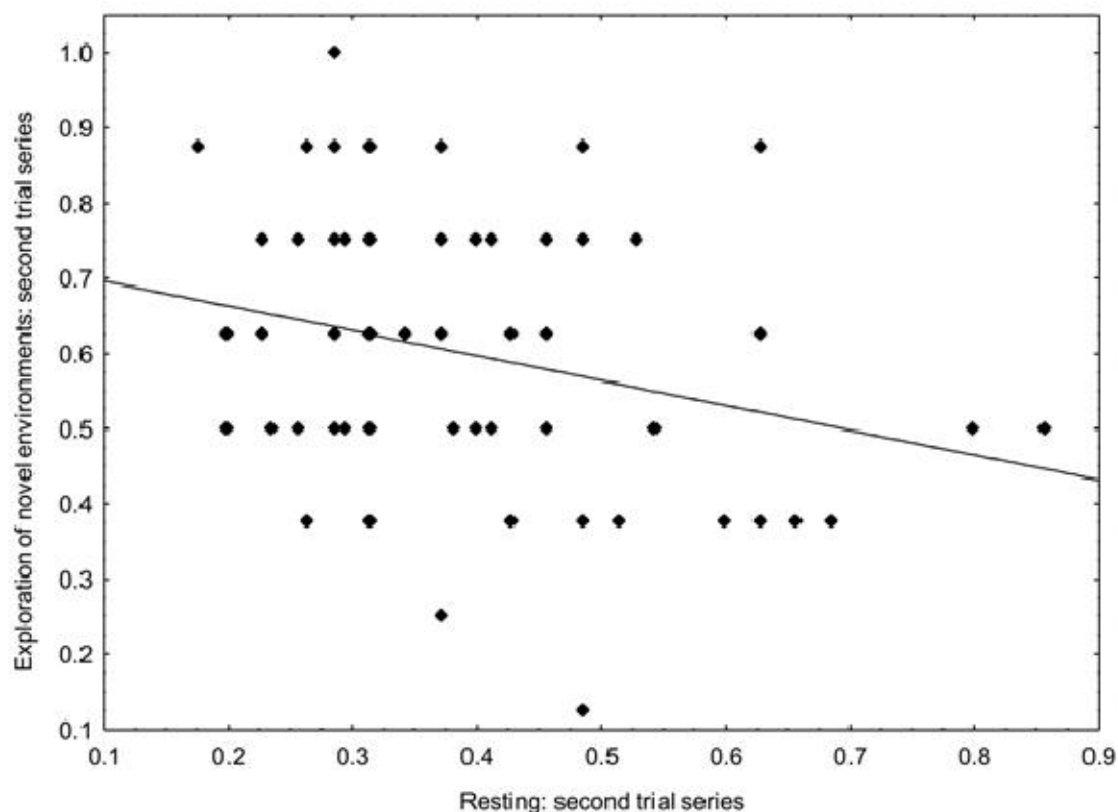
**Table 2.** Component loadings from PCA of the first trial series (~ 1 month after worker eclosion). Only components with an eigenvalue greater than one were extracted. All behavioral data, were included. Components were rotated using varimax rotation. Loadings larger than 0.32 are shown in bold.

Setting	Trait	PC 1	PC 2	PC 3	PC 4
Standardized experiments	Aggression	<b>0.341</b>	<b>0.726</b>	0.039	0.142
	Brood care	0.281	<b>-0.790</b>	0.063	0.151
	Exploration (object)	0.034	0.205	<b>-0.765</b>	0.004
	Exploration (environment)	0.010	-0.142	<b>-0.796</b>	0.041
Nest setting	Foraging	0.039	0.033	0.031	<b>-0.949</b>
	Resting	<b>0.680</b>	0.106	0.049	<b>0.422</b>
	Social care	<b>-0.881</b>	0.075	0.057	0.174
% variation explained		20.50	17.59	17.57	16.47

The second series of experiments (2-3 months after worker eclosion) resulted in four PCs explaining a total 72.1 % of the total behavioral variation (Table 3). PC 1 showed a negative association between resting behavior in the nest setting and both exploratory behaviors in the standardized experiments (Figure 2). The second PC indicated that workers that were interested in novel objects spent less time on brood care in the standardized experiments. Another negative relationship was uncovered by factor 3: workers performing more social care in the nest, spent less time on foraging. The fourth factor suggested that workers that behaved aggressively in the standardized experiments, spent less time on foraging in the nest context.

**Table 3.** Component loadings from PCA of the second trial series (2-3 months after worker eclosion). Only components with an eigenvalue greater than one were extracted. All behavioral data were included. Components were rotated using varimax rotation. Loadings larger than 0.32 are shown in bold.

Context	Trait	PC 1	PC 2	PC 3	PC 4
Standardized experiments	Aggression	0.041	0.053	-0.125	<b>-0.861</b>
	Brood care	0.065	<b>-0.924</b>	0.033	0.000
	Exploration (object)	<b>0.490</b>	<b>0.481</b>	0.259	-0.235
	Exploration (environment)	<b>0.839</b>	-0.060	-0.123	-0.168
Nest setting	Foraging	-0.081	0.091	<b>-0.568</b>	<b>0.529</b>
	Resting	<b>-0.756</b>	0.027	-0.269	-0.165
	Social care	0.039	0.019	<b>0.880</b>	0.133
% variation explained		21.84	15.72	18.12	16.43



**Figure 2.** Negative relationship of resting frequency (determined in the nest setting) and exploration of novel environments (measured in single ant experiments) of 66 same-aged individuals in the second trial series.



### **Influence of head width and ovary length**

The principal components of the first trial series were not significantly related to head width or ovary length. However, Pearson correlation revealed two trends indicating that mean ovary length was positively associated with PC 1 (Pearson:  $r = 0.21$ ,  $n = 66$ ,  $P < 0.10$ ) and PC 4 ( $r = 0.24$ ,  $P = 0.06$ ). Hence, workers with larger ovaries were tentatively more aggressive, rested more, but were less social (PC 1). In addition, they tentatively foraged less and again rested more (PC 4). Head width was unrelated to any PCs ( $P > 0.18$ ).

The second trial series gave two significant relationships: mean ovary length was positively correlated to PC 3 (Pearson:  $r = 0.34$ ,  $n = 66$ ,  $P < 0.01$ ) and negatively related to PC 4 ( $r = -0.26$ ,  $P = 0.04$ ). The positive correlation with PC 3 suggests that workers with larger ovaries perform more social care, but forage less. The negative correlation between PC 4 and mean ovary length indicates that workers with larger ovaries are more aggressive, but again forage less. Head width was again unrelated to any PCs ( $P > 0.11$ ).

### **Discussion**

Our study revealed that same-aged workers of the ant species *L. acervorum* clearly exhibit behavioral syndromes early in their life. Workers showed repeatability both in the nest setting (foraging and resting) and in the standardized experiments (exploration of novel environments). However, we also found changes in worker behavior with age: individuals became more active, foraged more and explored more novel environments. We found an overall exploration syndrome in both trial series demonstrating that workers that are fast in exploring novel environments are also more interested in novel objects. The second trial series demonstrated across-context consistency in behavior: individuals that were more active in the nest setting (i.e. rested less) were also more exploratory in standardized experiments (Table 3). Further, the PCAs indicate that aggressive workers are less active and social (first series), and also forage less (second series). Finally, we found that ovarian development influences foraging behavior and aggression: individuals with well-developed ovaries foraged less, but were more aggressive.

As predicted by our first hypothesis, we can demonstrate the classical temporal polyethism in *L. acervorum*. The findings from the scan samplings in the nest setting matched the expectations that the workers' activity and foraging frequency would rise over time. The fact, that workers also became more interested in discovering novel environments in individual trials, could be due to their increased activity levels and/or learning. Conversely,

other behaviors like aggression and brood care did not change with age. Sendova-Franks and Franks (1993) had shown that in the related *Temnothorax unifasciatus* (formerly *Leptothorax*) division of labor is only weakly age-related and more based on workers ‘foraging for work’: workers finding work in their current role will remain in their role, while workers repeatedly not finding work will move to a different task (Tofts and Franks 1992). Hence, even young workers could become dedicated foragers, while older ones remain nurses. In our study, at least three behavioral traits were influenced by age: foraging, resting and exploration of novel environments. In general, behavioral differences are probably due to a complex interplay of genetic, environmental and experiential factors, which are difficult to disentangle.

According to the response threshold models behavioral differences in the nest are due to differences in response thresholds among workers (reviewed in Beshers and Fewell 2001). An individual will only perform a task if the stimulus exceeds its internal threshold (Bonabeau et al. 1996). Different threshold values for distinct tasks within a colony may result from genetic differences (Gordon 1996). Surprisingly, the only repeatable behaviors were foraging, resting and exploration of novel environments. However, only foraging had a high latent repeatability score. Resting and exploration of novel environments had quite low repeatability scores indicating developmental effects or high plasticity.

In accordance with our second hypothesis we found several suites of correlated behaviors. The PCA revealed that aggressive workers undertake less brood and social care and in general rest more early in life. Later in life (second trial series) aggressive workers foraged less. Hence, this could characterize a distinct guard caste that rarely leaves the nest, does only little brood care or social care, but is highly aggressive. Our analyses also revealed an overall exploration syndrome between exploration of novel objects and environments. Workers that were very fast in discovering their surroundings also inspected more novel objects. Although this relationship might in part be due to the experimental set-up of testing both behaviors in one experiment, earlier experiments with *Temnothorax* workers showed that exploration of novel objects and environments do not always co-vary when tested in the same experiment (Modlmeier et al. 2012). A positive relationship between both exploratory behaviors has also been found in early exploratory behavior in male great tits (Verbeek et al. 1994). Accordingly, individuals were either quick and superficial, or slow and thorough explorers. As in our study these differences in exploratory behavior were not age-related. In addition, PCA suggests consistency across the different settings: exploratory workers in the individual experiments were also more active in the nest.

In accordance with our third hypothesis, we found an association between worker physiology and behavior. Body size in *L. acervorum* workers was unrelated to behavior. Many other studies have been able to find an association of behavior and morphology not only in polymorphic, but also in monomorphic species (e.g. Herbers 1983; Waser 1998; Fournier et al. 2007). Why we do not find an association of behavior and morphology is unclear, but possibly worker behavior in the small monomorphic colonies of *L. acervorum* is more influenced by the development of the ovaries, which are an important source of hormones in many insects (e.g. Dolezal et al. 2012). Indeed, our study demonstrated that workers with well-developed ovaries foraged less suggesting that foraging activity is influenced by ovarian activity. This is in line with numerous studies demonstrating that ovarian development leads to a specialization on tasks inside the nest (e.g. Bourke 1988; Fénéron et al. 1996; Pohl et al. 2011). Interestingly, division of labor between foragers and intranidal workers (that stay in the nest) was unrelated to age differences among workers in our study, as all observed individuals in our experiment were of similar age. Fénéron et al. (1996) had also demonstrated that physiological differences (i.e. different ovarian development) can lead to division of labor within an age class in ponerine ants.

In general, anatomical and physiological differences between the ovary size and the animal's behavior may depend on the workers age and on social interactions with other workers and the queen (Bonavita-Cougourdan and Morel 1985; Muscedere 2009). It is surprising that in our study workers with larger ovaries performed tentatively less social care early in life (first trial series), but more social care later on (second trial series). Further, mean ovary length was positively related to aggression in both trial series. In *Temnothorax allardycei* (formerly *Leptothorax*) high ranking workers (in queenright colonies) dominate co-workers (if necessary by fighting), have larger ovaries, lay more eggs and receive food from subordinates without reciprocation (Cole 1981). Accordingly, the formation of dominance hierarchies among freshly eclosed worker in *L. acervorum* could help explain our results: young workers striving for a higher rank will be more aggressive, perform less social care (will not feed lower ranking individuals) and have better developed ovaries. Conversely, Bourke (1991) found that fights between workers within a nest were not related to worker reproduction in *L. acervorum*. Further, a study on ant queens also revealed a strong link between individual aggressiveness and fertility status in polygynous nests (Ortius and Heinze 1999). If queens and workers follow a common 'ovarian ground plan' (as suggested by West-Eberhard 1996) these results could explain a positive relationship between aggression and fertility status in workers.

To conclude, we could show that there are clear behavioral differences between workers of the ant *Leptothorax acervorum*, which are consistent over time and across different settings. Albeit we found a general increase in activity, foraging and exploration with age, individuals showed high latent repeatability in foraging and rather low repeatability in resting and how fast they explored novel environments. A general exploration syndrome was present in both trial series indicating that exploration of novel environments and novel objects have a common regulatory basis. Across settings, a positive relationship was found between activity in the nest and exploration in standardized experiments. Our results also emphasize the importance of investigating physiological traits (like ovarian development) when studying behavioral syndromes. In our *L. acervorum* workers foraging was negatively correlated to ovary length. Hence, as shown in honeybees and other ant species ovarian development could be an important component regulating personalities and division of labor in ants.

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### **References**

- Amdam GV, Norberg K, Fondrk MK, Page RE. 2004. Reproductive ground plan may mediate colony-level selection effects on individual foraging behavior in honey bees. *Proc Natl Acad Sci USA*. 101:11350-11355.
- Bell AM. 2007. Future directions in behavioural syndromes research. *Proc Roy Soc B*. 274:755-761.
- Beshers SN, Fewell JH. 2001. Models of division of labor in social insects. *Annu Rev Entomol*. 46:413-440.
- Bonabeau E, Theraulaz G, Deneubourg JL. 1996. Quantitative study of the fixed threshold model for the regulation of division of labour in insect societies. *Proc Roy Soc B*. 263:1565-1569.
- Bonavita-Cougourdan A, Morel L. 1985. Polyethism in social interactions in ants. *Behav Process*. 11:425-433.
- Bourke AFG. 1988. Dominance orders, worker reproduction, and queen-worker conflict in the slave-making ant *Harpagoxenus sublaevis*. *Behav Ecol Sociobiol*. 23:323-333.
- Bourke AFG. 1991. Queen behaviour, reproduction and egg cannibalism in multiple-queen colonies of the ant *Leptothorax acervorum*. *Anim Behav*. 42:295-310.

- Buschinger A. 1974. Experimente und Beobachtungen zur Gründung und Entwicklung neuer Sozietäten der sklavenhaltenden Ameise *Harpagoxenus sublaevis* (Nyl.). *Insect Soc.* 21:381-406.
- Calabi P, Traniello JFA, Werner MH. 1983. Age polyethism: its occurrence in the ant *Pheidole hortensis*, and some general considerations. *Psyche.* 90:395-412.
- Chapman NC, Oldroyd BP, Hughes WOH. 2007. Differential responses of honeybee (*Apis mellifera*) patriline to changes in stimuli for the generalist tasks of nursing and foraging. *Behav Ecol Sociobiol.* 61:1185-1194.
- Chapman BB, Thain HT, Coughlin J, Hughes WOH. 2011. Behavioural syndromes at multiple scales in *Myrmica* ants. *Anim Behav.* 82:391-397.
- Cole BJ. 1981. Dominance hierarchies in *Leptothorax* ants. *Science.* 212:83-84.
- Comrey AL, Lee HB. 1992. A first course in factor analysis. Hillsdale, NJ: Lawrence Erlbaum Associates
- Dingemanse NJ, Dochtermann NA, Wright J. 2010. A method for exploring the structure of behavioural syndromes to allow formal comparison within and between data sets. *Anim Behav.* 79:439–450.
- Dolezal AG, Brent CS, Hölldobler B, Amdam GV. 2012. Worker division of labor and endocrine physiology are associated in the harvester ant, *Pogonomyrmex californicus*. *J Exp Biol.* 215:454-460.
- Dornhaus A. 2008. Specialisation does not predict individual efficiency in an ant. *PLoS Biology.* 6:2368-2375.
- Fénéron R, Durand JL, Jaisson P. 1996. Relation between behaviour and physiological maturation in a ponerine ant. *Behaviour.* 133:791-806.
- Fournier D, Battaille F, Timmermans I, Aron S. 2007. Genetic diversity, worker size polymorphism and division of labour in the polyandrous ant *Cataglyphis cursor*. *Anim Behav.* 75:151-158.
- Frumhoff PC, Baker J. 1988. A genetic component to division of labour within honey bee colonies. *Nature.* 333:358-361.
- Glennis EJ, Cahan S. 1999. Undertaking specialisation in the desert leaf-cutter ant *Acromyrmex versicolor*. *Anim Behav.* 58:437-442.
- Gordon DM. 1996. The organization of work in social insect colonies. *Nature.* 380: 121-124.
- Gordon DM, Guetz A, Greene MJ, Holmes S. 2011. Colony variation in the collective regulation of foraging by harvester ants. *Behav Ecol.* 22:429-435.
- Gotelli NJ, Ellison AM. 2004. A primer of ecological statistics. Sunderland, MA: Sinauer.
- Heinze J, Ortius D. 1991. Social organization of *Leptothorax acervorum* from Alaska (Hymenoptera: Formicidae). *Psyche.* 98:227-240.
- Herbers JM. 1983. Social organization in *Leptothorax longispinosus* Mayr. *Anim Behav.* 31:759-771.
- Hölldobler B, Wilson EO. 1990. The Ants. Cambridge, MA.: Harvard Univ. Press.
- Konrad M, Pamminger T, Foitzik S. 2012. Two pathways ensuring social harmony. *Naturwissenschaften.* Online first (DOI: 10.1007/s00114-012-0943-z).
- Mirenda JT, Vinson SB. 1979. A marking technique for adults of the red imported fire ant (Hymenoptera: Formicidae). *Fla Entomol.* 62:279-281.
- Modlmeier AP, Foitzik S. 2011. Productivity increases with variation in aggression among group members in *Temnothorax* ants. *Behav Ecol.* 22:1026–1032.
- Modlmeier AP, Liebmann JE, Foitzik S. 2012. Diverse societies are more productive: a lesson from ants. *Proc Roy Soc B.* 279:2142-2150.
- Muller H, Grossman H, Chittka L. 2010. ‘Personality’ in bumblebees: individual consistency in responses to novel colours? *Anim Behav.* 80:1065-1074
- Muscledere ML, Willey, TA, Traniello JFA. 2009. Age and task efficiency in the ant *Pheidole dentata*: young minor workers are not specialist nurses. *Anim Behav.* 77:911-918.

- Nakagawa S, Schielzeth H. 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol Rev.* 85:935-956.
- Oldroyd BP, Fewell JH. 2007. Genetic diversity promotes homeostasis in insect colonies. *Trends Ecol Evol.* 22:408-413.
- Ortius D, Heinze J. 1999. Fertility signaling in queens of a North American ant. *Behav Ecol Sociobiol.* 45:151-159.
- Pinter-Wollman N, Gordon DM, Holmes S. 2012. Nest site and weather affect the personality of harvester ant colonies, *Behav Ecol.* online first (DOI: 10.1093/beheco/ars066).
- Pohl S, Witte V, Foitzik S. 2011. Division of labor and slave raid initiation in slave-making ants. *Behav Ecol Sociobiol.* 65:2029-2036.
- R Development Core Team. 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ravary F, Lecoutey E, Kaminski G, Châline N, Jaisson P. 2007. Individual experience alone can generate lasting division of labor in ants. *Curr Biol.* 17:1308-1312.
- Robinson GE, Page RE. 1988. Genetic determination of guarding and undertaking in honeybee colonies. *Nature.* 333:356-358.
- Robinson GE. 1992. Regulation of division of labor in insect societies. *Annu Rev Entomol.* 37:637-665.
- Scharf I, Modlmeier AP, Fries S, Tirard C, Foitzik S. 2012. Characterizing the collective personality of ant societies: aggressive colonies do not abandon their home. *PLoS ONE:* 7:e33314.
- Seeley TD. 1982. Adaptive significance of the age polyethism schedule in honeybee colonies. *Behav Ecol Sociobiol.* 11:287-293.
- Sendova-Franks A, Franks NR. 1993. Task allocation in ant colonies within variable environments (a study of temporal polyethism, experimental). *B Math Biol.* 55:75-96.
- Siegel AJ, Kaftanoglu O, Fondrk MK, Smith NR, Page RE. 2012. Ovarian regulation of foraging division of labour in Africanized backcross and pollen-hoarding honeybees. *Anim Behav.* 83:653-658.
- Stamps J, Groothuis TGG. 2010. The development of animal personality: relevance, concepts and perspectives. *Biol Rev.* 85:301-325.
- Tofts C, Franks NR. 1992. Doing the right thing: ants, honeybees and naked mole-rats. *Trends Ecol Evol.* 7:346-349.
- Verbeek MEM, Drent PJ, Wiepkema PR. 1994 Consistent individual differences in early exploratory behaviour in male great tits. *Anim Behav.* 48:1113-1121.
- Waddington SJ, Santorelli LA, Ryan FR, Hughes WOH. 2010. Genetic polyethism in leaf-cutting ants. *Behav Ecol.* 21:1165-1169.
- Waser NM. 1998. Task-matching and short-term size shifts in foragers of the harvester ant, *Messor pergandei* (Hymenoptera: Formicidae). *J Insect Behav.* 11:451-462.
- West-Eberhard MJ. 1996. Wasp societies as microcosms for the study of development and evolution. In *Natural History and Evolution of Paper Wasps* (Turillazzi, S. and West-Eberhard, M.J., eds), Oxford University Press.
- Wilson EO. 1980. Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: Atta). *Behav Ecol Sociobiol.* 7:143-456.
- Wray MK, Mattila HR, Seeley TD. 2011. Collective personalities in honeybee colonies are linked to colony fitness. *Anim Behav.* 81:559-568.

## Chapter V: Characterizing the collective personality of ant societies: aggressive colonies do not abandon their home

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Workers of the ant species *Temnothorax nylanderi*.  
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This study was a joint effort of Inon Scharf (IS) and myself. The following contributions were equally shared between IS and myself: (i) conceiving and designing the experiment, (ii) collection of data and statistical analysis, (iii) supervising S. Fries (student project), and (iv) writing the manuscript, and (v) responding to reviewer comments

## Abstract

Animal groups can show consistent behaviors or personalities just like solitary animals. We studied the collective behavior of *Temnothorax nylanderii* ant colonies, including consistency in behavior and correlations between different behavioral traits. We focused on four collective behaviors (aggression against intruders, nest relocation, removal of infected corpses and nest reconstruction) and also tested for links to the immune defense level of a colony and a fitness component (per-capita productivity). Behaviors leading to an increased exposure of ants to micro-parasites were expected to be positively associated with immune defense measures and indeed colonies that often relocated to other nest sites showed increased immune defense levels. Besides, colonies that responded with low aggression to intruders or failed to remove infected corpses, showed a higher likelihood to move to a new nest site. This resembles the trade-off between aggression and relocation often observed in solitary animals. Finally, one of the behaviors, nest reconstruction, was positively linked to per-capita productivity, whereas other colony-level behaviors, such as aggression against intruders, showed no association, albeit all behaviors were expected to be important for fitness under field conditions. In summary, our study shows that ant societies exhibit complex personalities that can be associated to the physiology and fitness of the colony. Some of these behaviors are linked in suites of correlated behaviors, similar to personalities of solitary animals.



## Background

Variation in heritable traits such as morphology or behavior is expected to be constantly removed from natural populations by drift and natural or sexual selection and it is therefore interesting to study the factors that maintain variation (Brockmann 2001; Fitzpatrick 2009). Behavioral syndromes, defined as the consistency in behavior across different situations and contexts, can explain why behavioral variation is kept. The same behavior may be beneficial in certain contexts but may be maladaptive in other situations (e.g., Dall et al. 2004). This could result in non-directional selection on distinct behavioral types. For example, aggression can be useful against prey or competitors, but it can discourage or even lead to the premature death of potential mates, as it has been shown in a fishing spider (Johnson and Sih 2005). In addition, being active in the absence of predators may be beneficial, but high activity levels in their presence are often risky (Sih et al. 2004). Therefore, detecting correlations between behavioral traits is valuable for understanding how intra-population variation is maintained.

Behavioral syndromes have been described in various animal systems, with recurrent correlations between certain behaviors. A common behavioral syndrome is the aggressiveness-boldness syndrome. Aggressive individuals often tend to be more active and they take more risks (e.g., Sih et al. 2004; Moretz et al. 2007). For instance, aggressive male field crickets went faster out of refuge in a novel environment, that is, they were also bolder (Kortet and Hedrick 2007). Another common behavioral syndrome is related to activity in general. Many behaviors tend to be positively associated with the activity level of an individual, because they are simultaneously affected by the metabolic rate or time constraints (Moretz et al. 2007; Biro and Stamps 2010). This should lead to a triplet positive association among aggressiveness, boldness and general activity. Behavioral syndromes in solitary animals are often linked to physiological or life-history traits of the organism, which can help understanding the proximate correlates of behavior. For example, metabolic rate differences are a possible physiological explanation for consistent inter-individual differences in activity and aggression, explaining in part the aggressiveness-boldness-activity syndrome (Moretz et al. 2007; Biro and Stamps 2010).

While behavioral correlations are well documented for some solitary animals, there is still little evidence for behavioral correlations in animal societies (but see three recent papers: (Chapman et al. 2011; Gordon et al. 2011; Wray et al. 2011)). Using behavioral methodologies typical for solitary animals, these studies deal with research questions relevant to social insects, such as behavioral differences between castes or cooperative behavior of the whole colony. For example, Chapman et al. (2011) showed that the patroller caste in *Myrmica* ants

exhibited the common aggressiveness-boldness syndrome, while the brood-carer caste did not. In addition, the behavior of these two castes is correlated on the colony level (i.e., the whole colony is sometimes more aggressive and bold). Interestingly, social groups, as a whole, may differ in behavior, which can affect their success. Wray et al. (2011) showed that the defensive response of honey bee colonies was correlated with fitness components (the colony weight). In social insects, selection predominantly acts on the colony level and collective behaviors such as communal defense, networking in foraging and nest construction are expected to be strongly linked to colony productivity. Hence, colony behavior can be shaped by natural selection similar to the behavior of multicellular organisms.

We used colonies of the European cavity-dwelling ant, *Temnothorax nylanderi*, to study consistency, variation and the relationship of four important behaviors: (1) aggression towards an intruder, (2) nest relocation, (3) removal of an infected corpse, and (4) nest reconstruction after partial destruction. These behaviors represent important activities of animal societies in general. Collective defense is typical for many groups, and is evident in many bird species living in groups, as mobbing of predators gets more efficient with colony size (Krause and Ruxton 2002). In social insects, aggression is vital in defending the nest and has fitness consequences (e.g., Wray et al. 2011). Collective movement is an important trait of animal groups expressed by fish schools, bird flocks, locust swarms and social insects (reviewed in Sumpter 2006). Nest relocation is a common behavior in many social insect species, which is exhibited when the present nest becomes unsuitable for some reason, such as decomposition of the nesting material in cavity-dwelling ants (Pratt and Pierce 2001; Visscher 2007) or local food depletion in army ants (e.g., Schöning et al. 2005). Other reasons for nest relocation are reproduction – a large colony splits into two parts and one of them leaves, i.e., reproduction by budding (Visscher 2007) – and simply moving into a better-larger nest (Dornhaus et al. 2004).

Living in groups increases the parasite burden and the risk of infection by contact-transmitted parasites (Schmid-Hempel 1998; Krause and Ruxton 2002). Therefore, removal of waste and corpses of dead group members is crucial for colony health (Cremer et al. 2007). Indeed, waste management has been found in various group-living animals, which live in the same nest sites for longer time periods (e.g., aphids, mites and ants (Benton and Foster 1992; Sato and Saito 2006; Diez et al. 2011)). Nest sites provide a safe environment to raise offspring, but they have to be constructed and maintained, and failure to repair may lead to exposure to external risks, such as predators or parasites (Pike and Foster 2004). In order to increase defensiveness against intruders, colonies often block or reduce the nest entrance by

using soil, sand or wooden pieces (e.g., Seeley and Morse 1978; Herbers and Banschbach 1995; Pratt and Pierce 2001). Moreover, colonies of the ant genus *Temnothorax* prefer nest sites with very small entrances so that a single ant could control colony entry (Pratt and Pierce 2001).

Even before testing behavior under different conditions, testing for repeatability of behavior under the same conditions is a necessary step in characterizing behavioral syndromes and personality (Réale et al. 2007). Second, searching for collective colony personality, we looked for positive and/or negative correlations among the four behavioral traits. We predict that similar to the aggressiveness-boldness-activity syndrome in solitary animals, aggressive colonies should be bolder and more active. Therefore, they should show a better performance in the other collective behaviors such as nest reconstruction. However, as ant colonies are also energy limited we expect some behavioral trade-offs. In addition, it is intriguing to relate behavior and personality to fitness components (Réale et al. 2007; Smith and Blumstein 2008), and to understand whether different colony personalities result in the same final fitness. We therefore tested how the four behaviors correlate with per-capita productivity as a measure of colony efficiency (e.g., Michener 1964; Scharf et al. 2011). The four documented behaviors are important for colony survival, representing ways to overcome stress and threat. We expect in general a positive effect of the measured behaviors on per-capita productivity. However, we do not expect a perfect match, because the same goal can be achieved in parallel ways, and specializing in one behavior may lead to another becoming superfluous.

Immune defense is an important physiological trait in social insects, because frequent interactions of genetically similar individuals lead to a great risk of contagious infections (Schmid-Hempel 1998; Cremer et al. 2007). The level of immune defense may correlate with inter-colony differences in some behaviors, such as corpse removal and nest relocation. Encounters with infected corpses pose a direct threat to the colony, and the ants should react by increasing their immune defense (Diez et al. 2011). However, ant colonies that recognize and remove infectious material from the nest faster and thus show a high social immunity, might be able to invest less in the physiological immune defense. Nest relocation may be triggered by exposure to parasites, but during the move ants and their brood are also vulnerable to infection and predation (Cremer et al. 2007). Similarly, intruders might increase micro-parasite exposure (Schmid-Hempel 1998). We therefore expected that colonies which show a high tendency to expose their members to parasites either during nest migration, nest defense or by failing to reconstruct their nest site should invest highly in their immune

function. In contrast, ant colonies that remove infected corpses fast from the nest are expected to show low immune functions. Nest relocation should show the best positive correlation with the immune defense level, because during emigrations all colony members are exposed to the surrounding environment.

## **Materials and methods**

### **Study system**

*Temnothorax nylanderi* are small cavity-dwelling ants inhabiting forests in western and central Europe. Their colonies comprise several dozen workers and a single queen. They reside in preformed cavities of various wooden structures on the forest floor providing protection from the outer environment (Foitzik and Heinze 1998; Foitzik et al. 2003). Due to the decomposition of their nests in the field, these ant colonies are forced to frequently relocate their nests (Pratt and Pierce 2001; Dornhaus et al. 2004). *Temnothorax nylanderi* is a suitable species for the research questions because it shows high variability in behavior, mainly in relocation and aggressive tendencies. As mentioned, all studied behaviors have implications for the colony's performance under natural conditions. We collected 50 *T. nylanderi* colonies in summer 2009 in Sommerhausen, Germany (49.706N, 10.030E). No specific permits were required for the described ant collection (the collection site is not privately owned or protected and the collected ants are not endangered or protected). The colonies were brought to the laboratory and moved to artificial nests (7.5×2.5×0.5 cm) in plastic boxes (10×10×1.5 cm) with a plastered floor. The nests were kept in climate chambers imitating the natural temperatures around the year in the habitat of origin (summer: 20°/15°C day/night; autumn/spring: 15°/10°C day/night; winter: temperatures gradually decreased to -5°C). Colonies were fed weekly with honey, crickets and water. Colonies were kept for ~1.5 years under standard conditions to moderate environmental effects on behavior, physiology and life-history traits. Consequently, we estimate that more than 50% of the workers emerged in the laboratory. Two weeks prior to the experiment, colonies were moved to summer conditions. The experiments were conducted at room temperature.

### **Experimental design**

In March–May 2011 we tested the performance of the ant colonies in four standardized tests. We were interested in four important behaviors: (I) aggression against a conspecific intruder, (II) nest relocation, (III) corpse removal out of the nest, and (IV) reconstruction of the nest

after partial opening. Each behavior was tested twice for each colony, with one week in-between experiments. The behavioral observations were performed under stereomicroscopes by the two first authors, but all behaviors of a colony were observed by the same observer. In addition, we counted the number of workers (i.e., colony size) before the first behavioral test. After the aggression tests, we counted the number of brood items: New queen, male and worker pupae, pre-pupae and larvae and calculated the per-capita productivity for each colony (colony production divided by colony size). We were also interested in possible correlations of those four behaviors with the immune defense level.

### *I. Aggression against a conspecific intruder*

Colony aggression was quantified by entering a dead non-nestmate conspecific worker into the nest and measuring all aggressive interactions during the next five minutes (according to the protocol described in (Pamminger et al. 2011; Scharf et al. 2011)). Specifically, we documented every 20–30 seconds how many ants were either antennating or attacking the intruder (11 observations per trial). Dead ants, killed by freezing in  $-20^{\circ}\text{C}$  prior to the experiment, were used to eliminate behavioral variation between the stimuli and focus on the focal colony's response to the chemical stimulus. We used the percent of aggressive interactions (no. of mandible spreading, biting, dragging, holding and stinging events divided by all of the above plus antennating events), as a measure of aggression (Pamminger et al. 2011; Scharf et al. 2011). Aggression tests were performed twice for each colony (one week between the two trials).

### *II. Nest relocation*

On the day after each aggression test, we gave the ants the opportunity to relocate into new nests, without damaging the original nest. The new nest, which was identical to the original one, was placed seven centimeters from the occupied nest, and we noted after 24 hours whether the colonies relocated. It was a reasonable time, since after 24 hours ~40% of the colonies moved at least three workers to the new nest. We scored the relocation tendency according to four levels: No relocation (0), three or more workers without brood occur in the new nest (1), workers and brood occur in the new nest but still also occupy the old one, i.e., the colony has split (possible in *T. nylanderi*) (2), and complete relocation, the old nest is empty (3).

### *III. Removal of a corpse out of the nest*

Three weeks later we sacrificed a large *T. nylanderi* colony by freezing at  $-20^{\circ}\text{C}$ , opened its nest, added some water and let the ants decompose for 48 hours at room temperature ( $20^{\circ}\text{C}$ ). After that time the ant corpses were covered by white unidentified fungi (Fig. 1). We entered three ant corpses (randomly chosen) to each nest and measured the time the colony required to remove the first dead decomposed ant. We obtained the exact time of the corpse removal, if it was removed within the first 16 minutes. We took another observation after 30 minutes. If the corpse was removed after 16 minutes and before 30 minutes, the colony received the value “23 minutes”, and if no corpse was removed after 30 minutes, the colony received the maximal value of “30 minutes” (occurred in 5% of the cases). We repeated this procedure a week later.

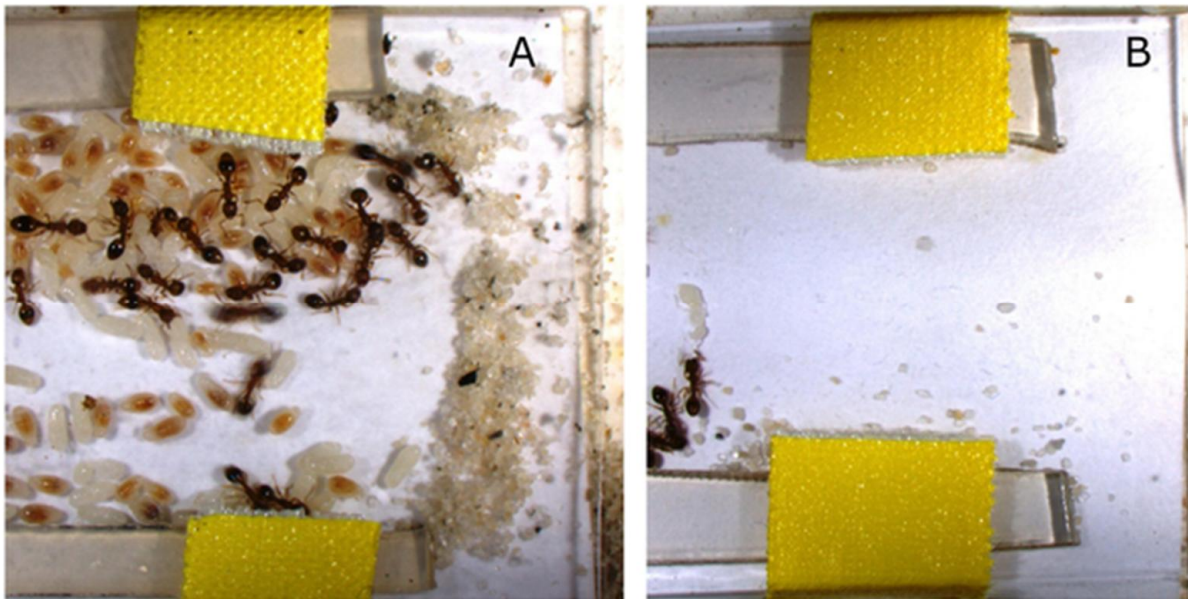


**Figure 1.** A decaying corpse of a *T. nylanderi* ant covered by unidentified fungi, which was used for the corpse removal experiment.

### *IV. Nest reconstruction after partial opening*

Two weeks after the corpse removal experiment we measured the last behavior, the reconstruction of the nest. We first removed parts of the plastic nest, so that the nest entrance was increased from 0.3 cm to 1.5 cm. We provided the ant nests with a standardized amount of fine sand (1 ml), which could be used by the workers to block part of the now widened nest

entrance. Previous experiments with *Temnothorax* ants have shown that these ants prefer nest sites with small entrances, which are easier to defend against larger ants (Pratt and Pierce 2001). Two days later we photographed the nest entrance and measured the percentage of the entrance which was covered with sand particles (Fig. 2): We drew a line closing the entrance and calculated the proportion of this line covered with sand. Digital measurements were done by the first author using the software ImageJ. We repeated the whole procedure a week later.



**Figure 2.** Two *T. nylanderii* nests representing two extremes of nest reconstruction after partial opening: (A) entrance is almost fully blocked again; (B) entrance is almost unblocked.

#### *V. Immune defense level*

After the behavioral experiments, we randomly chose 21 colonies and collected two foragers, two ants located next to the entrance and two ants taking care of the brood from each colony for immune defense measurements (a total of six ants, representing the colony immune defense level). We chose ants performing different tasks, as a previous study pointed to differences in immune defense among castes or workers performing different tasks (e.g., Bocher et al. 2007). As a proxy of immune defense level, we measured the activity of the phenoloxidase enzyme (PO). This enzyme is mainly found in the hemolymph as pro-phenoloxidase (PPO) and is activated into phenoloxidase prior to measurement. We therefore measured the total amount of PO potentially available for the individual (both activated and stored pro-enzyme). PO is an important component of invertebrates' immune defense and is involved in melanisation, cellular defense response and wound healing processes (Cerenius et al. 2008).

We used a similar procedure to Bocher et al. (2007). We placed individual ants in 20  $\mu\text{L}$  sodium cacodylate/ $\text{CaCl}_2$  buffer (0.01 M Na-Cac, 0.005 M  $\text{CaCl}_2$ ), cut them with small scissors, and centrifuged using a cooling device. We moved the liquid part to new Eppendorf tubes and added 10  $\mu\text{L}$  chymotrypsin to activate the PPO. As a substrate we used L-DOPA (4  $\text{mg mL}^{-1}$  in distilled water) and the reaction was performed at 30°C in a temperature-controlled spectrophotometer (Multiscan FC, Thermo Scientific, Vantaa, Finland) for 40 min. We measured the absorbance at 492 nm every 25 s. The enzyme activity was determined according to the slope of the linear phase of the reaction (200–675 s after the reaction's start). In each run of the spectrophotometer we used 3 negative controls. We removed cases in which the absorbance curve was too irregular. The amount of PPO may correlate with body size. In order to correct for that, each slope was divided by the head width of each ant, photographed prior to the PPO measurement using a digital camera and a binocular.

## Statistics

### *Consistency in behavior*

Prior to analysis, we used the Z-score transformation controlling for the variance of the variables in different units, i.e., subtracting the mean and dividing by the standard deviation (Gotelli and Ellison 2004), and the transformed values were used in all further statistical tests. In order to test for consistency in behavior of the first and second trial, we used three different tests: (1) The Individual Stability Statistic (hereafter, ISS; Asendorpf 1990):

$$ISS = 1 - \frac{(z_{t1} - z_{t2})^2}{2}, \text{ where } z_{t1} \text{ and } z_{t2} \text{ are the values of the two trials of each behavior; (2)}$$

The Cronbach's  $\alpha$ , measuring reliability of repeated tests (Wray et al. 2011; (3) Pearson correlation. Higher values in all three tests point to high consistency in behavior while lower values indicate higher variance. We then used the Pearson correlation to correlate among all four ISS values (a single value for each behavior tested, based on the two trials). The purpose was to detect whether colonies showing consistent behavior in one trait are also consistent in other traits (i.e., 'consistent in consistency'). It is plausible that colony size affects different behaviors and/or the level of consistency. In order to test for that, we correlated colony size with the four behaviors and the ISS levels.

### *Characterization of behavioral syndromes*

We used the raw data (not transformed) to calculate the mean of the two trials documented for every behavioral trait (note that means enabled intermediate levels of relocation behavior,



seven levels in total). Then we used the Z-score transformation on those mean values and performed a Factor Analysis (FA) on the four behavioral traits (Gotelli and Ellison 2004). We also applied a varimax rotation to facilitate interpretation. Time to dead conspecific removal was given in negative values, so fast removal will score highly, similar to high aggression and high levels of nest reconstruction (high values represent stronger expression of each behavior). FA is a common way to characterize behavioral syndromes (Dingemanse et al. 2010; Wray et al. 2011). The factors with eigenvalues larger than one should represent sets of behaviors. To further investigate these sets, we used a Pearson correlation.

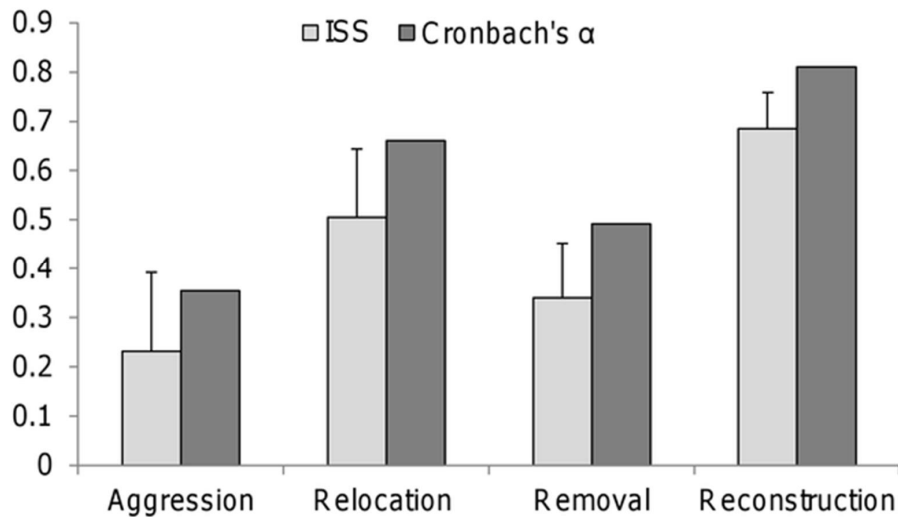
### *Linking behavior with productivity and immune defense*

We aimed at testing how the behavioral traits are linked to an important fitness component, the per-capita productivity. We already know that there is a negative link between colony size and per-capita productivity (Michener 1964). We therefore did not correlate the 1<sup>st</sup> and 2<sup>nd</sup> factors with per-capita productivity directly, but with the residuals of the linear regression of colony size and per-capita productivity (hereafter ‘productivity residuals’), in order to control for colony size. Then we used a two-way regression with the 1<sup>st</sup> and 2<sup>nd</sup> factors and their interaction as explanatory variables and the productivity residuals as the dependent variable. Regarding the immune defense level, we first tested whether colonies and ants performing different tasks differ in the PPO levels (a two-way ANOVA). Then, we used the average value of PPO for each colony (over six workers) and correlated the PPO levels with all four behavioral traits, using Bonferroni correction for multiple comparisons. We used STATISTICA v. 9.1 (StatSoft.Inc, Tulsa, OK, USA) and SYSTAT v. 11 (SYSTAT Software, San Jose, CA, USA) for all statistical analyses.

## **Results**

Consistency in behavior between the two trials, represented by the Individual Stability Statistic (ISS), was lower for aggressive behavior and corpse removal compared to nest reconstruction and relocation (Fig. 3). There was no correlation among consistencies ( $P > 0.26$  for all pairwise comparisons), meaning that there was no clear relationship among the consistency levels shown by colonies for different behavioral traits, i.e., high consistency in one behavior was not associated with high consistency in another one. When correlating pairs of trials separately for each behavior, significant positive correlations were evident for all behaviors except for aggression (aggression: Bartlett  $\chi^2$  statistic = 2.27, df = 1,  $P = 0.13$ ; nest relocation:  $\chi^2 = 13.36$ , df = 1,  $P = 0.0003$ ; corpse removal:  $\chi^2 = 5.38$ , df = 1,  $P = 0.020$ ; nest

reconstruction:  $\chi^2 = 29.14$ ,  $df = 1$ ,  $P < 0.0001$ ). Cronbach's  $\alpha$  tests produced similar results (Fig. 3), in accordance with the prior analysis using ISS values. Colony size was neither correlated with any of the observed behaviors ( $P > 0.32$ ) nor with behavioral consistency (ISS values;  $P > 0.39$ ), even without a Bonferroni correction, suggesting no link of colony size with the behaviors observed. Table 1 presents summary statistics for the two trials of the four behaviors observed.



**Figure 3.** Consistency values (ISS; bright grey, left) of the two behavioral trials for each of the four behaviors measured (means $\pm$ 1 SE), and Cronbach's  $\alpha$  coefficients of reliability tests (dark grey, right).

**Table 1.** Summary statistics for the two trials of the four behaviors observed.

Test	Mean $\pm$ 1 S.D.	Median
Aggression I	0.2401 $\pm$ 0.2821	0.1235
Aggression II	0.3282 $\pm$ 0.2528	0.2248
Nest relocation I	0.70 $\pm$ 0.95	0
Nest relocation II	0.76 $\pm$ 1.10	0
Corpse removal I	12.24 $\pm$ 8.29	10.51
Corpse removal II	10.22 $\pm$ 8.42	6.77
Nest reconstruction I	0.5295 $\pm$ 0.3107	0.5531
Nest reconstruction II	0.3197 $\pm$ 0.3179	0.2412

'I' and 'II' stand for the first and second trial.  
doi:10.1371/journal.pone.0033314.t001

We performed factor analysis (FA) on transformed means of the four behavioral traits. Eigenvalues, the percentage of the variance explained and factor loadings for different behaviors on all the FA axes are presented in Table 2.

**Table 2.** Results of the two first factors of the factor analysis performed on mean behaviors of two trials after applying a Z-score transformation and a varimax rotation.

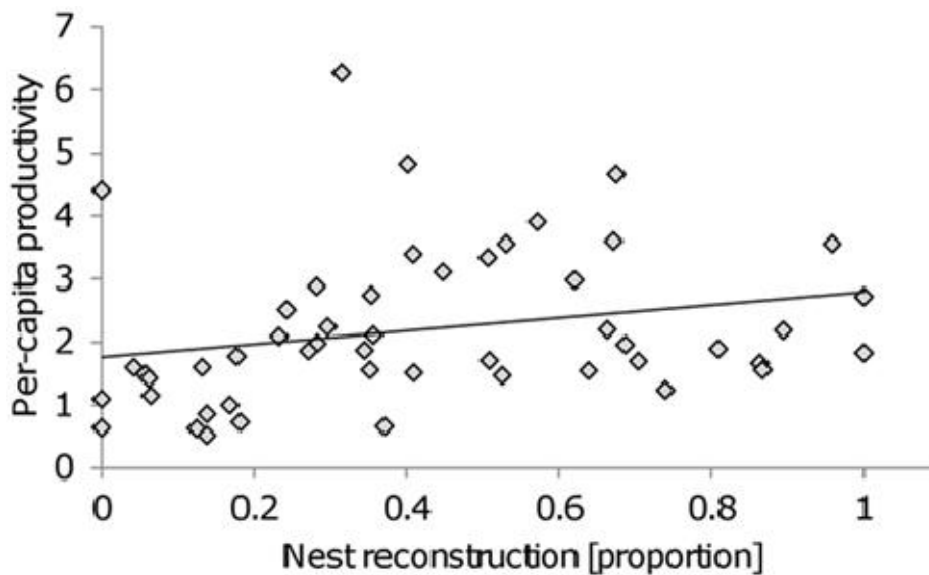
	<b>Factor 1</b>	<b>Factor 2</b>
Eigenvalue	1.51	0.97
% var. explained	36.1%	25.9%
Aggression	<b>+0.711</b>	+0.055
Nest relocation	<b>-0.749</b>	+0.083
Corpse removal	+0.614	+0.294
Nest reconstruction	-0.022	<b>-0.969</b>

Eigenvalues are taken from the unrotated analysis. Factor loadings higher than 0.7 are shown in bold. Factors 3 and 4 had lower Eigenvalues (0.78 and 0.74) and were not included.

doi:10.1371/journal.pone.0033314.t002

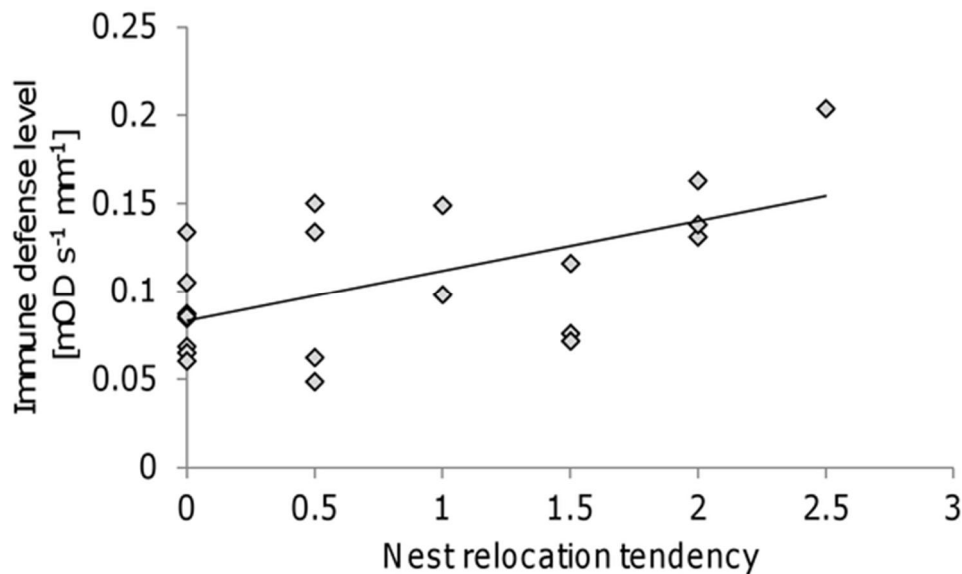
The 1<sup>st</sup> factor represented an ‘aggressive vs. emigration-prone’ colony personality, because aggression and nest relocation had high loadings on this factor, but in opposite signs (positive and negative respectively). Corpse removal loaded positively, similar to aggression but to a lesser extent. We performed a Pearson correlation between the untransformed means of the two main behaviors of the 1<sup>st</sup> factor, aggression and nest relocation, that confirmed to some extent the negative relationship (a marginally non-significant trend: Bartlett  $\chi^2$  statistic = 3.12, df = 1, P = 0.077). The 2<sup>nd</sup> factor was composed mainly of nest reconstruction (negative), while other behaviors showed loadings close to zero. The two other factors, the 3<sup>rd</sup> and 4<sup>th</sup> ones, had eigenvalues lower than 1 and are not further discussed. The 2<sup>nd</sup> factor negatively correlated with the productivity residuals (coefficient = -0.355, P = 0.019). It suggests a positive link between nest reconstruction and per-capita productivity (note that nest reconstruction scored negatively on the 2<sup>nd</sup> factor, and therefore a negative correlation of productivity with the 2<sup>nd</sup> factor implied on a positive correlation with nest reconstruction; Fig. 4). The 1<sup>st</sup> factor showed a marginally non-significant negative correlation with the productivity residuals (coefficient = -0.264, P = 0.077). It might suggest a negative effect of high aggression and a positive effect of relocation tendency on per-capita productivity, but

since it is not significant, this possible association is not further discussed. The interaction term was not significant ( $P = 0.94$ ) and was removed from analysis; statistics for the whole model are:  $F_{2,47} = 4.59$ ,  $R^2 = 0.164$ ,  $P = 0.015$ . In summary, the 1<sup>st</sup> factor showed a trade-off between aggression and corpse removal vs. nest relocation, while the 2<sup>nd</sup> factor was mainly composed of nest reconstruction. The correlation of the 2<sup>nd</sup> factor with productivity suggests that reconstruction contributes to this fitness component, but a manipulation is required to better support this association.



**Figure 4.** The positive relationship between nest reconstruction (proportion) and per-capita productivity (total number of brood divided by number of workers). The trend line is the best-fitted line according to a linear regression test.

Immune defense levels, represented by phenol- and pro-phenoloxidase (PPO), were evenly distributed among ants performing different tasks ( $F_{2,56} = 0.51$ ,  $P = 0.60$ ), but differed between colonies ( $F_{20,56} = 2.08$ ,  $P = 0.017$ ). The two-way interaction term was not significant ( $F_{40,56} = 1.08$ ,  $P = 0.39$ ). PPO levels were positively correlated with the relocation tendency (Pearson coefficient = 0.59,  $P = 0.020$ ; Fig. 5), that is colonies which tended to relocate their nests show higher immune defense levels. All other behavioral traits were not correlated with PPO levels after Bonferroni correction for multiple comparisons.



**Figure 5.** The positive relationship between the nest relocation tendency and the immune defense level, corrected for body size (head width of individual ants). The trend line is the best-fitted line according to a linear regression test.

## Discussion

Behavioral syndromes and personalities/temperament have often been demonstrated for solitary animals, but evidence for syndromes in insect societies or characterization of collective personality are still rare (but see (Chapman et al. 2011; Gordon et al. 2011; Wray et al. 2011)). Our study is one of the first to show collective personality on the colony level. The most important result is the evidence for a collective personality: colonies that defend their nest, either by fighting against intruders more aggressively or by removing infected corpses more efficiently, are less likely to relocate after a disturbance. It fits a common trade-off between competitiveness and emigration tendencies (e.g., Zirkle et al. 1988; Korona 1989). The behavioral consistency was the highest for nest reconstruction and relocation, less strong for removal of corpses and non-significant for aggression. This difference is probably related to the level of specialization each activity requires. Interestingly, the immune defense level was correlated with the nest relocation tendency, but with no other behavior, possibly because nest emigration is the only action exposing the whole colony to the surrounding environment. Finally, there is a positive correlation between per-capita productivity and nest reconstruction, suggesting a link between behavior and a fitness component.

The trade-off between territory defense, either by defending against intruders (elevated aggression) or parasites (efficient corpse removal), and the tendency to relocate is often shown by solitary animals. We suggest several explanations for this trade-off in ant societies. First, some nests might be considered to be of better quality than others and were therefore

fiercer defended and less easily abandoned. *Temnothorax* colonies easily distinguish between nest types and often move if a better nest is available (Pratt and Pierce 2001; Dornhaus et al. 2004). Second, defending the nest implies that ants invested effort and energy, and therefore are reluctant to move out. Third, after colonies fail for some reason to fight back against intruders or to remove potential source of infection, they tend to relocate more readily to another nest, which might be more defensible. This trade-off between defense against intruders and relocation tendency has parallels in solitary animals owning a territory. For example, Cichlid fish males tended less to abandon their territory in the presence of predators if they were defending it before the encounter with predators (Markert and Arnegard 2007), and less aggressive Cichlid females were more likely to emigrate than more aggressive ones (Schradin and Lamprecht 2002). Yet, the aggressiveness-relocation trade-off shown here also fits a more general ecological pattern: more competitive animals usually stay while less competitive ones emigrate, e.g., flour beetles (Zirkle et al. 1988; Korona 1989). In related *T. longispinosus* colonies, aggressive colonies were more often found in dense areas (Modlmeier and Foitzik 2011). This suggests that aggressive colonies remain in these dense areas and do not relocate despite frequent disturbances by intruders while less aggressive ones may move away.

The consistency in behavior was higher for nest reconstruction and relocation than aggression and removal of corpses. The two latter behaviors are performed by specialist ants, while the two former are a true collective behavior of the whole colony. Therefore, the repeatability of behaviors based on only a few specialist ants is possibly weaker, because specialist ants could have died between trials or on the other hand can also improve their efficiency with trials. Similarly, corpse removal in other social insects is done by few specialists, to minimize the exposure to contagious elements. This behavior may vary according to response thresholds to corpse/waste removal (Cremer et al. 2007; Waddington and Hughes 2010; Diez et al. 2011). Similarly, aggression and kin discrimination are presumably carried out by a small group of specialist workers (Crosland 1990), leading to the same pattern of low consistency in behavior. In comparison, nest relocation requires a more coordinated effort: In a related *Temnothorax* species, one third of the colony recruits nestmates, actively participating in nest relocation (Pratt et al. 2002). We believe that the link between worker specialization and the consistency in colony behavior is important for further understanding behavioral syndromes in social insects. An interesting future direction would be to increase environmental heterogeneity and look for behavioral consistencies, expecting

that consistency would be negatively correlated with environmental heterogeneity in space or time (Stamps and Groothuis 2010).

The immune defense level was positively correlated with the tendency of nest relocation, but not with any other behavior. Further research is required to establish more firmly the relation between immune defense level and relocation tendency, also in interaction with other environmental factors. We suggest that nest relocation is the only tested behavior exposing all colony members to the external environment, while the three other behaviors are carried out by a small fraction of the colony workers. Specialized workers can prevent the exposure of the whole colony to external risks in such cases, but exposure is inevitable during emigration. We suggest that colonies try to behaviorally adjust to the risk, but when not possible, react physiologically.

The relationship between different behaviors and fitness components is often taken for granted but is a fundamental issue in behavioral ecology (i.e., behavior is assumed to optimize fitness). Specifically in the field of behavioral syndromes and animal personality, there is a need for a better link with fitness (Sih and Johnson 2005; Réale et al. 2007). Smith and Blumstein (2008) reviewed fitness consequences of animal personality, and showed that exploration was positively correlated with animal survival, and aggression increased with reproductive success. In social insects, Wray et al. (2011) showed a link between foraging and defense behaviors with productivity and survival, and Modlmeier and Foitzik demonstrated a positive relationship between the within-colony variance in behavior and productivity in the field (2011) and in the laboratory (Modlmeier et al. 2012). We showed here a possible link between the nest reconstruction behavior and productivity.

Social insect colonies often prefer small entrances to their nests, as small entrances are more easily defensible (e.g., Pratt and Pierce 2001). Nest usurpation of *Temnothorax* colonies is a common phenomenon, often by other ant species of larger colonies (e.g., Yamaguchi 1992). Presumably to avoid invasions of different natures, *Temnothorax* species often reduce the entrance further more by accumulating dirt particles (Herbers and Banschbach 1995). Other social insect species close their entrance in various ways in order to protect the colony against invasions (Seeley and Morse 1978; Cerdá and Retana 1998). But to the best of our knowledge, this is the first study showing a positive correlation of nest reconstruction with some fitness component. Such a link can result from the positive contribution of this behavior to fitness or alternatively may indicate that colonies having more brood relative to workers block their entrance more intensively, because they have more to lose from invasions to their nests than colonies with less brood. The other observed behaviors did not correlate with per-

capita productivity. A possible reason may be that after a while in lab conditions, behaviors which enhance survival and productivity under field conditions become less relevant. In general, measuring fitness of insect societies is challenging, because of their complex life cycle and long life span. It is also plausible that colonies would employ different short- and long-term strategies, resulting in various effects on fitness components (Scharf et al. 2011). Finally, a more experimental approach would be to investigate the suggested trade-off of aggression with nest relocation. Manipulating the nest condition/quality or alternatively inducing different levels of aggression can be a promising approach.

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### **Author contributions**

Conceived and designed the experiments: IS, APM, S. Foitzik. Performed the experiments: IS, APM, S. Fries. Analyzed the data: IS, APM, S. Foitzik. Contributed reagents / materials / analysis tools: CT, S. Foitzik. Wrote the paper: IS, APM, S. Foitzik. Established the immunological analyses for the study species: CT.

### **References**

- Asendorpf JB. 1990. The measurement of individual consistency. *Methodika*. 4:1–23.
- Benton TG, Foster WA. 1992. Altruistic housekeeping in a social aphid. *Proc Roy Soc B*. 247:199–202.
- Biro PA, Stamps JA 2010. Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends Ecol Evol*. 25:653–659.
- Bocher A, Tirard C, Doums C. 2007. Phenotypic plasticity of immune defence linked with foraging activity in the ant *Cataglyphis velox*. *J Evol Biol*. 20:2228–2234.
- Brockmann HJ. 2001. The evolution of alternative strategies and tactics. *Advances in the Study of Behavior*. 30:1–51.
- Cerdá X, Retana J. 1998. Interference interactions and nest usurpation between two subordinate ant species. *Oecologia*. 113:577–583.
- Cerenius L, Lee BL, Söderhäll K. 2008. The proPO-system: pros and cons for its role in invertebrate immunity. *Trends in Immunology*. 29:263–271.
- Chapman BB, Thain H, Coughlin J, Hughes WOH 2011. Behavioural syndromes at multiple scales in *Myrmica* ants. *Anim Behav*. 82:391–397.
- Cremer S, Armitage SAO, Schmid-Hempel P 2007. Social immunity. *Curr Biol*. 17:R693–R702.
- Crosland MWJ. 1990. Variation in ant aggression and kin discrimination ability within and between colonies. *J Insect Behav*. 3:359–379.
- Dall SRX, Houston AI, McNamara JM. 2004. The behavioral ecology of personality: consistent individual differences from an adaptive perspective. *Ecol Lett*. 7:734–739.
- Diez L, Deneubourg JL, Hoebcke L, Detrain C 2011. Orientation in corpse-carrying ants: memory or chemical cues? *Anim Behav*. 81:1171–1176.



- Dingemanse NJ, Dochtermann N, Wright J. 2010. A method for exploring the structure of behavioural syndromes to allow formal comparison within and between data sets. *Anim Behav.* 79:439–450.
- Dornhaus A, Franks NR, Hawkins RM, Shere HNS 2004. Ants move to improve: colonies of *Leptothorax albipennis* emigrate whenever they find a superior nest site. *Anim Behav.* 67:959–963.
- Fitzpatrick BM, Shook K, Izally R. 2009. Frequency-dependent selection by wild birds promotes polymorphism in model salamanders. *BMC Ecology.* 9:12.
- Foitzik S, Heinze J. 1998. Nest site limitation and colony takeover in the ant *Leptothorax nylanderi*. *Behav Ecol.* 9:367–375.
- Foitzik S, Strätz M, Heinze J. 2003. Ecology, life history and resource allocation in the ant, *Leptothorax nylanderi*. *J Evol Biol.* 16:670–680.
- Gordon DM, Guetz A, Greene MJ, Holmes S 2011. Colony variation in the collective regulation of foraging by harvester ants. *Behav Ecol.* 22:429–435.
- Gotelli NJ, Ellison AM. 2004. A primer of ecological statistics. Sunderland, MA, USA: Sinauer.
- Herbers JM, Banschbach V. 1995. Size-dependent nest site choice by cavity-dwelling ants. *Psyche.* 102:13–17.
- Johnson JC, Sih A. 2005. Precopulatory sexual cannibalism in fishing spiders (*Dolomedes triton*): a role for behavioral syndromes. *Behav Ecol Sociobiol.* 58:390–396.
- Korona R. 1989. Evolutionarily stable strategies in competition for resource intake rate maximization: II. Oviposition behavior in *Tribolium confusum*. *Behav Ecol Sociobiol.* 25:201–205.
- Kortet R, Hedrick A 2007. A behavioural syndrome in the field cricket *Gryllus integer*: intrasexual aggression is correlated with activity in a novel environment. *Biol J Linn Soc.* 91:475–482.
- Krause J, Ruxton GD 2002. Living in groups. Oxford, UK: Oxford University Press.
- Markert JA, Arnegard ME. 2007. Size-dependent use of territorial space by a rock-dwelling cichlid fish. *Oecologia.* 154:611–621.
- Michener CD. 1964. Reproductive efficiency in relation to colony size in hymenopterous societies. *Insect Soc.* 4:317–341.
- Modlmeier AP, Foitzik S. 2011. Productivity increases with variation in aggression among group members in *Temnothorax* ants. *Behav Ecol.* 22:1026–1032.
- Modlmeier AP, Liebmann JE, Foitzik S. 2012. Diverse societies are more productive: a lesson from ants. *Proc Roy Soc B.* 279:2142–2150.
- Moretz JA, Martinus EP, Robison BD 2007. Behavioral syndromes and the evolution of correlated behavior in zebrafish. *Behav Ecol.* 18:556–562.
- Pamminger T, Scharf I, Pennings PS, Foitzik S. 2011. Increased host aggression as an induced defense against slave-making ants. *Behav Ecol.* 22:255–260.
- Pike N, Foster W 2004. Fortress repair in the social aphid species *Pemphigus spyrothecae*. *Anim Behav.* 67:909–914.
- Pratt SC, Mallon EB, Sumpter DJT, Franks NR. 2002. Quorum sensing, recruitment, and collective decision-making during colony emigration by the ant *Leptothorax albipennis*. *Behav Ecol Sociobiol.* 52:117–127.
- Pratt SC, Pierce NE 2001. The cavity-dwelling ant *Leptothorax curvispinosus* uses nest geometry to discriminate between potential homes. *Anim Behav.* 62: 281–287.
- Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. *Biol Rev.* 82:291–318.
- Sato Y, Saito Y. 2006. Nest sanitation in social spider mites: interspecific differences in defecation behavior. *Ethology.* 112:664–669.

- Scharf I, Bauer S, Fischer-Blass B, Foitzik S. 2011. Impact of a social parasite on ant host populations depends on host species, habitat and year. *Biol J of Linn Soc.* 103:559–570.
- Scharf I, Pamminger T, Foitzik S. 2011. Differential response of ant colonies to intruders: attack strategies correlate with potential threat. *Ethology.* 117:731–739.
- Schmid-Hempel P 1998. Parasites in social insects. Princeton, NJ, USA: Princeton University Press.
- Schöning C, Njagi WM, Franks NR 2005. Temporal and spatial patterns in the emigrations of the army ant *Dorylus (Anomma) molestus* in the montane forest of Mt Kenya. *Ecol Entomol.* 30:532–540.
- Schradin C, Lamprecht J. 2002. Causes of female emigration in the group-living Cichlid fish *Neolamprologus multifasciatus*. *Ethology.* 108:237–248.
- Seeley TD, Morse RA. 1978. Nest site selection by the honey bee, *Apis mellifera*. *Insect Soc.* 25:323–337.
- Sih A, Bell A, Johnson JC 2004. Behavioral syndromes: an ecological and evolutionary overview. *Trends Ecol Evol.* 19:372–378.
- Smith BR, Blumstein DT. 2008. Fitness consequences of personality: a meta-analysis. *Behav Ecol.* 19:448–455.
- Stamps J, Groothuis TGG. 2010. The development of animal personality: relevance, concepts and perspectives. *Biol Rev.* 85:301–325.
- Sumpter DJT 2006. The principles of collective animal behaviour. *Philos Trans Roy Soc B.* 361:5–22.
- Visscher PK. 2007. Group decision making in nest-site selection among social insects. *Annu Rev Entomol.* 52:255–275.
- Waddington SJ, Hughes WHO. 2010. Waste management in the leaf-cutting ant *Acromyrmex echinator*: the role of worker size, age and plasticity. *Behav Ecol Sociobiol.* 64:1219–1228.
- Wray MK, Mattila HR, Seeley TD 2011. Collective personalities in honeybee colonies are linked to colony fitness. *Anim Behav.* 81:559–568.
- Yamaguchi T. 1992. Interspecific interference for nest sites between *Leptothorax congruus* and *Monomorium intrudens*. *Insect Soc.* 39:117–127.
- Zirkle DF, Dawson PS, Lavie B. 1988. An experimental analysis of the genetic relationships among life-history traits and emigration behavior in *Tribolium castaneum*. *Oikos.* 53:391–397.

## General discussion

My dissertation sheds light on the evolution of social behavior and provides empirical evidence that behaviorally diverse societies are more productive supporting theoretical models on the benefits of within-group variation. Further, we show that the concept of animal personalities can be applied to ants, both on the individual and on the colony level. Moreover, we describe ecological factors that select for distinct behavioral types and describe physiological mechanisms that underlie behavioral variation among individuals. In summary, the results of my dissertation show that it is vital to study multiple organizational levels and to examine both proximate and ultimate causes for the maintenance of behavioral variation to better understand the evolution of social behavior.

### **How do ecological factors shape variation in behavior?**

The question why animals behave the way they do and how evolution shapes behavior in response to ecological or social conditions has long been a central aspect of behavioral ecology. In short scientists in this research field want to gain a “functional understanding of behavior” (Westneat and Fox 2010). One behavior might be favored in a specific situation, while it could lead to fitness costs in another. In female great tits, for example, aggression is only favored in a competitive situation (Dingemanse et al. 2004). We investigated whether personality traits were associated with an important fitness measure (i.e. colony productivity) in ants by taking into account the ecological conditions and thus the selective environment. Our results revealed a positive association of aggression, population density and productivity. How can we explain the positive relationship between aggression and population density? According to classic quantitative genetics, the measured phenotype is influenced by genetic and environmental effects (Wolf and Moore 2010). Hence, higher aggression in dense areas could be due to natural selection (more aggressive colonies outcompete less aggressive ones) and/or phenotypic plasticity in behavior in response to environmental effects. The latter can be partitioned into nonsocial and social environmental effects (Wolf and Moore 2010). Nonsocial effects that increase aggression could be frequent interactions with opponents as in Argentine ants (Thomas et al. 2007; Van Wilgenburg et al. 2010) or encounters with parasites. A recent study by Pamminer et al. (2011) demonstrated short-term behavioral plasticity due to nonsocial environmental effects: *Temnothorax longispinosus* colonies were able to increase their aggression level for several days after contact with the slavemaking ant *P. americanus*.

Conversely, our field manipulation (chapter 3) did not reveal any long-term nonsocial environmental effects in response to changes in the parasite presence or population density. The observed consistency across worker generations indicates genetic effects and/or an influence of the social environment. Albeit we did not study the impact of the social environment on aggressiveness, this is a promising area for future research. According to a recent review by Bergmüller and Taborsky (2010), the social environment is a key factor for the evolution of animal personalities and its dynamics can be compared to predator-prey and host-parasite interactions, because social behaviors are under selection and selective agents at the same time. Irrespective of the mechanism, aggressive colonies appear to be favored in densely populated areas (for a possible behavioral mechanism see the aggression-relocation trade-off in chapter 5). The question is: why are not all colonies as aggressive as possible? High aggression may be maladaptive in less dense areas, because as a byproduct of aggression nest mates are occasionally attacked leading to fitness costs. Similarly, inappropriate behaviors were found in salamander larvae under sunfish predation (Sih et al. 2003). Hence, environmental heterogeneity and/or fluctuations might have important consequences for the maintenance of behavioral variation.

### **Parasite-host interaction favors aggressive host personalities**

Barber and Dingemans (2010) suggested that parasitism (as an ecological factor) could strongly influence host behavior and the evolution of divergent personality traits. In this context, the coevolutionary arms race between brood parasites (i.e. “social parasites” in the social insect literature) and their hosts is especially suited to study the relationship of differences in host personality and parasitism (Avilés and Parejo 2011). Both avian brood parasites and social parasites exploit the cooperative brood care of another species to raise their young. It is therefore not surprising that the coevolutionary arms race between host and parasite has many parallels in birds and social insects (see Kilner and Langmore 2010). At an early stage of such a coevolutionary arms race, selection on attack and defense traits can be very similar to a classical predator-prey arms race (Kilner and Langmore 2010). Slavemaking ants, which in fact can be considered as “micropredators” (Brandt et al. 2005), and their hosts exhibit an ideal model system to investigate the influence of parasitism on host personality, because their interactions are characterized by violence and overt aggression, i.e. strongly influenced by behavioral interactions (Foitzik et al. 2001, Brandt and Foitzik 2004). The result of our study that aggressive colonies better protect their brood against parasitic invasion, can therefore not only be seen in the light of parasite-host, but also of predator-prey

coevolution. Similar to our study, aggressive nest defense behavior has been demonstrated to increase offspring production in birds, e.g. in female Ural Owls (Kontiainen et al. 2009). From the parasite perspective, the hostile takeover of the first host colony by slavemaking founding queens is a crucial event in the life cycle of this social parasite. A failure to obtain enough (future) slaves at this point in life could result in the death of the parasite queen as the slaves perform almost all the work. Hence, parasites not only exert an intense selection pressure on host behavior during this time of the year, but are themselves strongly influenced by the behavior of the host. Fluctuations in parasite occurrence could help to maintain behavioral variation in host aggressiveness, especially if higher aggression leads to strong net costs in unparasitized areas. In summary, our study is a prime example for the influence of parasitism on host personality and stresses the importance of coevolutionary arms races for the evolution of animal personality differences.

### **Benefits of within-group variation**

Natural selection should reduce variation through directional selection and genetic drift and thereby “optimize” the trait under selection. In social groups, however, within-group variation itself could be a trait under selection, because selection also acts on the group level in highly structured societies (Korb und Heinze 2004). The results of my dissertation demonstrate that behaviorally diverse groups are more productive. This supports the theoretical framework of “social heterosis” by Nonacs and Kapheim (2007, 2008) and is in accordance with a recent study by Pruitt and Riechert (2011) demonstrating that higher within-group variation increases fitness in social spiders. Hence, our results suggest that the benefits of within-group variation could be in part responsible for the omnipresent ecological success of social groups. Indeed, comparing different subsocial and social spider species revealed that the evolution of sociality is accompanied with an adaptive increase in behavioral variation in spiders (Pruitt et al. 2012). Furthermore, behavioral variation contributes to division of labor, one of the key components for the success of social insect societies (Wilson 1987). From an evolutionary perspective, behavioral specialization constitutes the first step towards complex superorganisms like the leafcutter ants, in which behavioral task specialization is accompanied by morphological specialization (Wilson 1980).

### **Physiological aspects of worker personality and division of labor**

While it is important to study the ultimate reasons for the maintenance of within-group variation, it can be equally valuable to examine the proximate mechanisms that lead to the

observed variation among group members and moreover to the division of labor. In particular, physiological processes have been demonstrated to influence behavior in numerous species (see Stamps and Groothuis 2010). Within the social insects most castes are indeed rather physiologically than genetically determined (Oster and Wilson 1978). Our study revealed that physiological aspects were related to behavioral differences: e.g. foragers possessed less developed ovaries than inside workers. These results are in accordance with an earlier study by Fénéron et al. (1996) showing that division of labor among workers of the same age cohort was influenced by ovarian development in the ponerine ant, *Ectatomma tuberculatum*. Both our study and the study by Fénéron et al. (1996) support the “ovarian ground plan” suggested by Mary Jane West-Eberhard (1996): workers and queens follow a common regulatory ground plan, in which individuals with developed ovaries stay inside the nest to take care of the brood and individuals with undeveloped ovaries leave the nest to forage or scout.

The differences in ovarian development in *L. acervorum* workers could not only result in indirect fitness consequences, but also influence individual fitness directly, because workers occasionally produce males in this species. Although aggressive interactions among workers of the same nest occur and presumably represent dominance interactions (as in other lepto thoracine species; Cole 1981), aggression could not be linked to worker reproduction so far (Bourke 1991). Our study revealed that workers with larger ovaries were more aggressive, but the question remains how good our measure of worker aggression (against a frozen non-nestmate) represents aggression against nestmates. If it reliably predicts aggression versus nestmates, our study would suggest that high worker aggression is related to the reproductive state, presumably through dominance interactions. Therefore, the evolution of personalities may be linked to and/or restricted by physiological traits.

### **Collective personality**

Although experiments with single individuals can yield interesting results in the context of within-group variation and help to explain the mechanisms that underlie variation, it cannot measure their level of cooperation. Moreover, in self-organizing systems the collective pattern can be “more than the sum of its parts”, because the output can be increased through positive feedback (Sumpter 2006). Therefore, the study of emergent actions of the group as a whole can give us important insights into group-level selection and allows us to measure overall group performance. By testing several collective behaviors in *T. nylanderii* colonies, we were able to demonstrate consistent differences in colony behavior, i.e. collective personalities, as previously found in honey bees (Wray et al. 2011) and other ant species (e.g. harvester ants;

Gordon et al. 2011). Interestingly, nest reconstruction was positively related to productivity. Hence, nest reconstruction could be a proxy for overall collective performance or just be related to other traits that make a colony more efficient. Further, Franks and Deneubourg (1997) suggested that the brood cluster serves as a mechanical template that determines where the nest wall should be built. Hence, having less brood might result in less effective wall building. We also found a negative relationship between colony aggression and nest relocation, which represents a common trade-off between competitiveness and emigration tendency in solitary organisms (e.g. Korona 1989). This observed linkage could be in part responsible for the positive relationship between aggression and density (chapter 1). Finally, immune defense levels were positively related to relocation tendency indicating that immunological challenged colonies are more likely to relocate and/or that frequent relocation increases exposure to parasites and/or stress. More experiments are needed to disentangle cause and effects between immune level and relocation tendency. Links between behavioral traits and immune traits could have severe fitness consequences and should therefore be studied in a pace-of-life syndrome (Réale et al. 2010) that considers covariation of behavioral, immunological, physiological and life-history traits to investigate why certain traits are favored in a specific environment.

## **Future directions**

### *Manipulation of group composition*

While our correlative studies on the benefits of within-group variation in the field (chapter 1) and the laboratory (chapter 2) were an important first step, it is hard to evaluate cause and effects purely based on correlations. Therefore, the next logical step should be to manipulate the group composition by creating groups of known behavioral distribution (as suggested by Pinter-Wollman 2012). These groups could then be used to study the influence of group composition on the performance of collective behaviors like nest relocation or nest defense. Further, these artificial groups should then be analyzed in regard to their productivity, both in the field and the laboratory. This approach could yield insights into the cause and effects of within-group behavioral variation.

### *Early experience and development of worker personality*

Another issue concerns the heritability of personality traits and the influence of early experience on the development of personalities. Our studies indicate that aggressiveness is an ideal candidate to study the genetic basis of behavior. While we had already shown that

aggression remains consistent over several months despite the emergence of new workers, we cannot rule out that developmental effects or in this case the social environment contributed to this consistency. A cross-over experiment placing pupae (shortly before emergence) of known origin into colonies of known behavioral distribution could help to disentangle genetic from developmental effects. Further, exposing workers early in life to competitors/enemies, starvation and temperature fluctuations could shed light on the development of individual worker personalities, especially when combined with physiological analyses of their ovarian development.

*Fitness consequences of collective personalities in the field*

A promising approach would be to continue the study of collective personalities in the field. Our laboratory study on *T. nylanderi* colonies already demonstrated consistency in several behaviors. Hence, it would be intriguing to look for fitness consequences in a natural environment. Colonies could be kept in a field enclosure during the reproductive season and only shortly brought to the laboratory for census and behavioral experiments. The use of wire markings (of the queens) and field enclosures might allow observations over several seasons and hence a study on long-term effects of personality differences. Thereby, other fitness measures like winter survival could also be investigated in terms of personality.



## References

- Achenbach A, Foitzik S. 2009. First evidence for slave rebellion: enslaved ant workers systematically kill the brood of their social parasite *Protomognathus americanus*. *Evolution*. 63:1068-1075.
- Avilés JM, Parejo D. 2011. Host personalities and the evolution of behavioural adaptations in brood parasitic-host systems. *Anim Behav*. 82:613-618.
- Barber I, Dingemanse NJ. 2010. Parasitism and the evolutionary ecology of animal personality. *Phil Trans R Soc B*. 365:4077-4088.
- Bergmüller R, Taborsky M. 2010. Animal personality due to social niche specialisation. *Trends Ecol Evol*. 25:504-511.
- Beshers SN, Fewell JH. 2001. Models of division of labor in social insects. *Annu Rev Entomol*. 46:413-440.
- Boag PT, Grant PR. 1981. Intense natural selection in a population of Darwin's finches (Geospizinae) in the Galápagos. *Science*. 214:82-85.
- Bonabeau E, Theraulaz G, Deneubourg JL. 1996. Quantitative study of the fixed threshold model for the regulation of division of labour in insect societies. *Proc R Soc Lond B*. 263:1565-1569.
- Bourke AFG. 1991. Queen behaviour, reproduction and egg cannibalism in multiple-queen colonies of the ant *Leptothorax acervorum*. *Anim Behav*. 42:295-310.
- Brandt M, Foitzik S. 2004. Community context and specialization influence coevolution between a slavemaking ant and its hosts. *Ecology*. 85:2997-3009.
- Brandt M, Foitzik S, Fischer-Blass B, Heinze J. 2005. The coevolutionary dynamics of obligate ant social parasite systems – between prudence and antagonism. *Biol Rev*. 80:251-267.
- Buschinger A. 1968. Mono- und Polygynie bei Arten der Gattung *Leptothorax* Mayr (Hymenoptera: Formicidae). *Insect Soc*. 15:217-225.
- Calabi P, Traniello JFA, Wernger MH. 1983. Age polyethism: its occurrence in the ant *Pheidole hortensis*, and some general considerations. *Psyche*. 90:395-412.
- Cole, BJ. 1981. Dominance hierarchies in *Leptothorax* ants. *Science*. 212:83-84.
- Darwin C. 1859. *On the origin of species*. London: John Murray.
- Dingemanse NJ, Both C, Drent PJ, Tinbergen JM. 2004. Fitness consequences of avian personalities in a fluctuating environment. *Proc R Soc B*. 271:847-852.

- Dingemanse NJ, Both C, Drent PJ, Van Oers K, Van Noordwijk AJ. 2002. Repeatability and heritability of exploratory behaviour in great tits from the wild. *Anim Behav.* 64:929-938.
- Dornhaus A. 2008. Specialisation does not predict individual efficiency in an ant. *PLoS Biol.* 6:2368-2375.
- Fénéron R, Durand JL, Jaisson P. 1996. Relation between behaviour and physiological maturation in a ponerine ant. *Behaviour.* 133:791-806.
- Foitzik S, Achenbach A, Brandt M. 2009. Locally adapted social parasite affects density, social structure, and life history of its ant hosts. *Ecology.* 90:1195-1206.
- Foitzik S, DeHeer CJ, Hunjan DN, Herbers JM. 2001. Coevolution in host-parasite systems: behavioral strategies of slave-making ants and their hosts. *Proc R Soc B.* 268:1139-1146.
- Foitzik S, Heinze J. 1998. Nest site limitation and colony take over in the ant, *Leptothorax nylanderi*. *Behav Ecol.* 9:367-375.
- Foitzik S, Heinze J. 2000. Intraspecific parasitism and split sex ratios in a monogynous and monandrous ant (*Leptothorax nylanderi*). *Behav Ecol Sociobiol.* 47:424-431.
- Foitzik S, Heinze J. 2001. Microgeographic genetic structure and intraspecific parasitism in the ant *Leptothorax nylanderi*. *Ecol Entomol.* 26:449-456.
- Franks NR, Deneubourg J-L. 1997. Self-organizing nest construction in ants: individual worker behavior and the nest's dynamics. *Anim Behav.* 54:779-796.
- Franks NR, Tofts C. 1994. Foraging for work: how tasks allocate workers. *Anim Behav.* 48:470-472.
- Gordon D. 1996. The organization of work in social insect colonies. *Nature.* 380:121-124.
- Gordon D, Guetz A, Greene MJ, Holmes S. 2011. Colony variation in the collective regulation of foraging by harvester ants. *Behav Ecol.* 22:429-435.
- Gosling SD. 2001. From mice to men: what can we learn about personality from animal research? *Psychol Bull.* 127:45-86.
- Grant PR, Grant BR, Smith JNM, Abbott IJ, Abbott LK. 1976. Darwin's finches: population variation and natural selection. *Proc Nat Acad Sci USA.* 73:257-261.
- Heinze J, Foitzik S, Hippert A, Hölldobler B. 1996. Apparent dear-enemy phenomenon and environment-based recognition cues in the ant *Leptothorax nylanderi*. *Ethology.* 102:510-522.

- Heinze J. 2006. Life in a nutshell – social evolution in formicoxenine ants. In: Life cycles in social insects: behaviour, ecology and evolution (Kipyatkov VE, ed.). St. Petersburg: St. Petersburg University Press.
- Herbers JM. 1983. Social organization in *Leptothorax longispinosus* Mayr. Anim Behav. 31:759-771.
- Herbers JM, Stuart RJ. 1996. Multiple queens in ant nests: impact on genetic structure and inclusive fitness. Am Nat. 147:161-187.
- Hölldobler B, Wilson EO. 1990. The ants. Cambridge, MA: Belknap Press.
- Hölldobler B, Wilson EO. 2009. The superorganism: the beauty, elegance and strangeness of insect societies. New York, NY: W.W. Norton and Co.
- Hunt J, Hodgson D. 2010. What is fitness, and how do we measure it? In: Evolutionary Behavioral Ecology (Westneat DF, Fox CW, eds). New York, NY: Oxford Univ. Press.
- Johnson JC, Sih A. 2005. Precopulatory sexual cannibalism in fishing spiders (*Dolomedes triton*): a role for behavioral syndromes. Behav Ecol Sociobiol. 58:390-396.
- Jones JC, Myerscough MR, Graham S, Oldroyd BP. 2004. Honey bee nest thermoregulation: diversity promotes stability. Science. 305:402-404.
- Kilner RM, Langmore NE. 2011. Cuckoos versus hosts in insects and birds : adaptations, counter-adaptations and outcomes. Biol Rev. 86 :836-852.
- Kontiainen P, Pietäinen H, Huttunen K, Karell P, Kolunen H, Brommer JE. 2009. Aggressive Ural owl mothers recruit more offspring. Behav Ecol. 20:789-796.
- Korb J, Heinze J. 2004. Multilevel selection and social evolution of insect societies. Naturwissenschaften. 91:291-304.
- Korona R. 1989. Evolutionary stable strategies in competition for resource intake rate maximization: II. Oviposition behavior in *Tribolium confusum*. Behav Ecol Sociobiol. 25:201-205.
- Modlmeier AP, Foitzik S. 2011. Productivity increases with variation in aggression among group members in *Temnothorax* ants. Behav Ecol. 22:1026-1032.
- Mousseau TA, Roff DA. 1987. Natural selection and the heritability of fitness components. Heredity. 59:181-197.
- Myerscough MR, Oldroyd BP. 2004. Simulation models of the role of genetic variability in social insect task allocation. Insect Soc. 51:146-152.
- Nonacs P, Kapheim KM. 2007. Social heterosis and the maintenance of genetic diversity. J Evol Biol. 20:2253-2265.

- Nonacs P, Kapheim KM. 2008. Social heterosis and the maintenance of genetic diversity at the genome level. *J Evol Biol.* 21:631-635.
- Oster GF, Wilson EO. 1978. *Caste and ecology in the social insects.* Princeton, NJ: Princeton University Press.
- Pamminger T, Scharf I, Penningers PS, Foitzik S. 2011. Increased host aggression as an induced defense against slave-making ants. *Behav Ecol.* 22:255-260.
- Pinter-Wollman N. 2012. Personality in social insects: How does worker personality determine colony personality? *Curr Zool.* 58:579-587.
- Pruitt JN, Oufiero CE, Avilés L, Riechert S. 2012. Iterative evolution of increased behavioral variation characterizes the transition to sociality in spiders and proves advantageous. *Am Nat.* 180:496-510.
- Pruitt JN, Riechert SE. 2011. How within-group behavioural variation and task efficiency enhance fitness in a social group. *Proc R Soc B.* 278:1209-1215.
- Radchenko A. 2004. A review of the ant genera *Leptothorax* Mayr and *Temnothorax* Mayr (Hymenoptera, Formicidae) of the Eastern Palaearctic. *Acta Zool Acad Sci H.* 50:109-137.
- Ravary F, Lecoutey E, Kaminski G, Châline N, Jaisson P. 2007. Individual experience can generate lasting division of labor in ants. *Curr Biol.* 17:1308-1312.
- Réale D, Festa-Bianchet M. 2003. Predator-induced natural selection on temperament in bighorn ewes. *Anim Behav.* 65:463-470.
- Réale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio P-O. 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. *Phil Trans R Soc B.* 365:4051-4063.
- Réale D, Reader SM, Sol D, McDougall, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. *Biol Rev.* 82:291-318.
- Robinson GE. 1992. Regulation of division of labor in insect societies. *Annu Rev Entomol.* 37:637-665.
- Seeley T. 1982. Adaptive significance of the age polyethism schedule in honeybee colonies. *Behav Ecol Sociobiol.* 11:287-293.
- Seifert B. 2007. *Die Ameisen Mittel- und Nordeuropas.* Görlitz/Tauer: Iutra – Verlags- und Vertriebsgesellschaft.
- Sih A, Bell A, Johnson JC. 2004. Behavioral syndromes: an ecological and evolutionary overview. *Trends Ecol Evol.* 19:372-378.

- Sih A, Bell A, Johnson JC. 2010. Behavioral syndromes. In: Evolutionary Behavioral Ecology (Westneat DF, Fox CW, eds). New York, NY: Oxford Univ. Press.
- Sih A, Kats LB, Maurer EF. 2003. Behavioural correlations across situations and the evolution of antipredator behaviour in a sunfish-salamander system. *Anim Behav.* 65:29-44.
- Smith BR, Blumstein DT. 2008. Fitness consequences of personality: a meta-analysis. *Behav Ecol.* 19:448-455.
- Spencer H. 1866. The principles of biology. New York, NY: D. Appleton and Company.
- Stamps J, Groothuis TGG. 2010. The development of animal personality: relevance, concepts and perspectives. *Biol Rev.* 85:301-325.
- Sumpter DJT. 2006. The principles of collective animal behaviour. *Phil Trans R Soc B.* 361:5-22.
- Thomas ML, Payne-Makrisâ CM, Suarez AV, Tsutsui ND, Holway DA. 2007. Contact between supercolonies elevates aggression in Argentine ants. *Insectes Soc.* 54:225-233.
- Van Wilgenburg E, Clémencet J, Tsutsui ND. 2010. Experience influences aggressive behaviour in the Argentine ant. *Biol Lett.* 6:152-155.
- West-Eberhard MJ. 1996. Wasp societies as microcosms for the study of development and evolution. In *Natural History and Evolution of Paper Wasps* (Turillazzi, S. and West-Eberhard, M.J., eds). Oxford University Press.
- Westneat DF, Fox, CW. Preface. In: Evolutionary Behavioral Ecology (Westneat DF, Fox CW, eds). New York, NY: Oxford Univ. Press.
- Wheeler WM. 1911. The ant-colony as an organism. *J Morphol.* 22:307-325.
- Wilson DS. 1998. Adaptive individual differences within single populations. *Phil Trans R Soc B.* 353:199-205.
- Wilson EO. 1980. Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*). *Behav Ecol Sociobiol.* 7:143-456.
- Wilson EO. 1987. Causes of ecological success: the case of the ants. *J Anim Ecol.* 56:1-9.
- Wolf JB, Moore AJ. 2010. Interacting phenotypes and indirect genetic effects. In: Evolutionary Behavioral Ecology (Westneat DF, Fox CW, eds). New York, NY: Oxford Univ. Press.
- Wray MK, Mattila HR, Seeley TD. 2011. Collective personalities in honeybee colonies are linked to colony fitness. *Anim Behav.* 81:559-568.
- Yerkes RM. 1939. The life history and personality of the chimpanzee. *Am Nat.* 73:97-112.