The Emergence and Scaling of Division of Labor in Insect Societies

by

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A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved April 2011 by the
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May 2011

ABSTRACT

Division of labor, whereby different group members perform different functions, is a fundamental attribute of sociality. It appears across social systems, from simple cooperative groups to complex eusocial colonies. A core challenge in sociobiology is to explain how patterns of collective organization are generated. Theoretical models propose that division of labor self-organizes, or emerges, from interactions among group members and the environment; division of labor is also predicted to scale positively with group size. I empirically investigated the emergence and scaling of division of labor in evolutionarily incipient groups of sweat bees and in eusocial colonies of harvester ants.

To test whether division of labor is an emergent property of group living during early social evolution, I created *de novo* communal groups of the normally solitary sweat bee *Lasioglossum* (*Ctenonomia*) NDA-1. A division of labor repeatedly arose between nest excavation and guarding tasks; results were consistent with hypothesized effects of spatial organization and intrinsic behavioral variability. Moreover, an experimental increase in group size spontaneously promoted higher task specialization and division of labor.

Next, I examined the influence of colony size on division of labor in larger, more integrated colonies of the harvester ant *Pogonomyrmex californicus*. Division of labor scaled positively with colony size in two contexts: during early colony ontogeny, as colonies grew from tens to hundreds of workers, and among same-aged colonies that varied naturally in size. However, manipulation of colony size did not elicit a short-term response, suggesting that the scaling of division of labor in *P. californicus* colonies is a product of functional integration and underlying developmental processes, rather than a purely emergent epiphenomenon. This research provides novel insights into the organization of work in insect societies, and raises broader questions about the role of size in sociobiology.

To my wife, Cecily, whose love sustains me.

ACKNOWLEDGMENTS

First and foremost, I thank my advisor, Jennifer Fewell, for being an excellent mentor, motivator, and friend. I am also deeply grateful to my committee members, Jürgen Gadau, Jon Harrison, Bert Hölldobler, and Bob Johnson, for their invaluable insight, inspiration, and encouragement.

This work was made possible by intellectual and technical contributions from many assistants and collaborators, including, but not limited to, Raisa Ahmad, Ruhani Alam, Phil Barden, Antonio Benavidez, Sue Bertram, Rebecca Clark, Ti Dang, Adam Dolezal, Leah Drake, Amber Dunning, Root Gorelick, Dina Grayson, Bill Griffin, Kevin Haight, Jennifer Hale, Emma Harrison, Raphaël Jeanson, Penny Kukuk, Kurt Licence, Jürgen Liebig, Peter Lillywhite, Dakota Lippincott, Dani Moore, Rick Overson, Clint Penick, Stephen Pratt, Kimberly Shaffer, Zach Shaffer, Adam Siegel, Adrian Smith, Brian Smith, Tom Taylor, and James Waters. I was further stimulated by the Social Insect Research Group and the Center for Social Dynamics and Complexity. The SOLS Graduate Programs staff was very helpful. Finally, I thank my parents for their formative life lessons and unwavering support.

This dissertation was supported by the National Science Foundation (Graduate Research Fellowship to C.T.H., Doctoral Dissertation Improvement Grant IOS-1011180 to C.T.H. and J.H.F., and IOS-0446415 to J.H.F.), the ASU Graduate & Professional Student Association (research and travel grants), and the ASU School of Life Sciences (Completion Research Assistantship, summer fellowships, and travel awards).

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CHAPTER 1

INTRODUCTION

Division of labor is a fundamental property of biological systems across all levels of organization, from cells to societies (Maynard Smith and Szathmáry 1995). In social groups, division of labor is broadly defined as a statistical pattern whereby different individuals perform different functions, even if only temporarily (Michener 1974; Fewell et al. 2009). More precisely, individual group members preferentially perform specific tasks instead of distributing their effort evenly across available tasks, and each task is performed by a subset of the group (Gorelick et al. 2004).

Division of labor occurs to varying degrees across social systems and taxa, including insects, fish, birds, and mammals (Fetherston et al. 1990; Stander 1992; Lacey and Sherman 1997; Underwood and Shapiro 1999; Clutton-Brock et al. 2003; Arnold et al. 2005; Gazda et al. 2005; Ridley and Raihani 2008; Bruintjes and Taborsky 2010). However, it is most developed and best studied in eusocial insects such as ants, termites, and some bees and wasps (Wilson 1971; Michener 1974; Hölldobler and Wilson 1990, 2009). Eusocial colonies, by definition, exhibit reproductive division of labor between egg-laying queens and functionally sterile workers. Moreover, non-reproductive tasks such as brood care, nest maintenance, and foraging are often further subdivided among workers. Division of labor is thought to enhance individual- and colony-level performance (Oster and Wilson 1978; Dornhaus 2008; Chittka and Muller 2009), and is therefore considered to be of paramount importance to the ecological success of eusocial insects, which are among the most abundant and dominant animals on earth (Wilson 1971; Hölldobler and Wilson 1990).

Self-organization of division of labor

A core challenge in sociobiology is to explain how collective patterns of organization such as division of labor are generated. In many social systems, including insect colonies, global patterns arise from local, nonlinear interactions among group members and the environment, without central or external control (Bonabeau et al. 1997; Camazine et al. 2001; Hemelrijk 2002; Sumpter 2006). This process is called self-organization, and the resulting patterns are emergent properties. Emergent properties cannot be predicted by simply summing individual behaviors. They are produced via positive and negative feedback responses that amplify and stabilize behavioral changes, respectively.

How might division of labor self-organize? A number of emergence models have been proposed, mostly in the context of eusociality (reviewed by Beshers and Fewell 2001). Response threshold models posit that an individual is more likely to perform a given task when the external stimulus it encounters exceeds its internal response threshold (Robinson and Page 1989; Bonabeau et al. 1996, 1998; Page and Mitchell 1998). If individuals vary in their response thresholds for different tasks, and if the performance of a task reduces the intensity of an associated stimulus, then a division of labor can emerge in which workers with the lowest thresholds for a given task become specialists for that task. Consistent with the basic assumptions of the response threshold model, interindividual variation in task propensity has a genetic component in eusocial and other cooperative systems (Costa and Ross 2003; Oldroyd and Fewell 2007; Smith et al. 2008).

An extension of the response threshold model, termed self-reinforcement, proposes that thresholds are modified by experience: successful performance of a task lowers an individual's corresponding threshold, increasing the probability that it will continue performing that task. Conversely, unsuccessful performance or lack of opportunity to perform a task raises an individual's threshold, reducing the probability of future performance (Deneubourg et al. 1987; Plowright and Plowright 1988; Theraulaz et al. 1998). Self-reinforcement is thus capable of generating division of labor within an initially homogeneous group.

Although it has not yet been fully incorporated into emergence models, spatial organization may also contribute to division of labor. If task stimuli or opportunities are unevenly distributed in space, then spatial sorting of individuals may expose different individuals to different tasks, leading to specialization (Tofts and Franks 1992; Sendova-Franks and Franks 1995; Robinson et al. 2009). Spatial segregation, and subsequent task differentiation, can be induced by agonistic interactions in social systems with (Powell and Tschinkel 1999; Robson et al. 2000) or without (Hart and Ratnieks 2001; O'Donnell 2001) reproductive dominance.

Division of labor in evolutionarily incipient social groups

There are other plausible causes of division of labor, depending on the social structure, ecology, and phylogeny of the species; however, the response threshold model and spatial effects may be generally applicable across diverse social systems. In fact, their prerequisite conditions could theoretically be met in any group, perhaps even at the evolutionary origins of sociality (Page 1997; Page and Mitchell 1998; Fewell and Page 1999). The emergence of division of labor early in social evolution could provide a novel substrate for natural selection to act upon as groups evolve.

To test for self-organization of division of labor in newly-formed social groups, Fewell and colleagues established foundress associations of normally haplometrotic (solitary founding) *Pogonomyrmex* ant queens; group members differentiated into excavation and brood care specialists (Fewell and Page 1999; Helms Cahan and Fewell 2004; Jeanson and Fewell 2008). As predicted by the response threshold model, the individual that performed more excavation when solitary became the excavation specialist in pairs (Fewell and Page 1999; Helms Cahan and Fewell 2004). Furthermore, some haplometrotic populations exhibited higher levels of division of labor than pleometrotic (cooperative founding) populations, suggesting that the evolution of stable cooperative groups may favor task generalization or sharing (Fewell and Page 1999; Helms Cahan and Fewell 2004; but see Jeanson and Fewell 2008). Although all ants are eusocial, these studies of foundress associations imply that division of labor can emerge at the origins of sociality.

Lasioglossum: a model for the early evolution of division of labor

Lasioglossum (Halictidae: Halictinae) is a cosmopolitan genus of mostly ground-dwelling sweat bees; it is also among the most socially labile of all insect taxa (Michener 1974; Wcislo 1997a; Schwarz et al. 2007). Females of some species exhibit predominantly solitary life-histories, while others are facultatively or obligately eusocial, with reproductive division of labor. Intermediate in size and complexity, but not representing a transitional stage (Kukuk 1992; Schwarz et al. 2007; Wcislo and Tierney 2009), are communal groups in which adult females share a nest, with each provisioning her own offspring. This variability makes *Lasioglossum* an excellent model for comparative studies of social organization and evolution (Wcislo 1997a; Schwarz et al. 2007).

Building on the earlier work in ant foundress associations, but employing a system with no known history of social evolution, Jeanson et al. (2005) created 'communal' groups of normally solitary *Lasioglossum* (*Ctenonomia*) NDA-1 females; again, a division of labor arose, this time between nest excavation and guarding the nest entrance. Response threshold variation and spatial segregation were both implicated as potential underlying mechanisms, but direct support was lacking. Moreover, their experimental design could not determine whether division of labor was truly an emergent property (i.e., the degree of task differentiation should be greater than expected due to random variation). In a follow-up experiment that I co-designed and executed, but which is not included in my dissertation, self-reinforcement was ruled out as a primary cause of division of labor in this incipient social system (Jeanson et al. 2008).

Chapters 2 and 3 extend this line of research by investigating whether and how division of labor self-organizes in *de novo* groups of *L*. NDA-1. Chapter 2 also tests for a fitness cost of excavation specialization, which could select against division of labor as communal groups evolve (Helms Cahan and Fewell 2004; Jeanson et al. 2005). In addition, Chapter 3 addresses whether division of labor spontaneously increases with group size, as predicted by response threshold models (Gautrais et al. 2002; Merkle and Middendorf 2004; Jeanson et al. 2007) and spatial dynamics, if larger groups are more spatially heterogeneous.

Social scaling

As animal societies increase in size during development and/or evolution, they can undergo major behavioral and functional reorganization (Elgar 1989; Tschinkel 1993; Bourke 1999; Anderson and McShea 2001; Jeanne 2003; Yip et al. 2008; Pollard and Blumstein 2008, 2011; Hou et al. 2010; Waters et al. 2010). We use the term ‘social scaling’ to refer to predictable changes in individual- or group-level properties in response to changes in group size. Social scaling is analogous to biological scaling at other levels of organization; body size, in particular, has profound consequences for the structure, function, and ecology of organisms (Peters 1983; Schmidt-Nielsen 1984; Brown and West 2000).

What are the underlying causes of social scaling relations? Group-size effects may be emergent byproducts of self-organization (Gautrais et al. 2002; Merkle and Middendorf 2004; Jeanson et al. 2007); the scaling of division of labor in newly-formed associations of sweat bees is a potential example (above;

Chapter 3). But in more integrated, evolutionarily derived social groups, scaling patterns could also reflect functional constraints and/or adaptive responses to selection (Bourke 1999; Anderson and McShea 2001; Bonner 2004; Pollard and Blumstein 2008, 2011).

Eusocial insects are prime candidates for social scaling. First, they exhibit tremendous variation in colony size. Among the social Hymenoptera (ants, bees, and wasps), colonies range in size by many orders of magnitude, from tens of workers to tens of millions (Wilson 1971; Michener 1974; Hölldobler and Wilson 1990; Kaspari and Vargo 1995). Colony size varies during ontogeny as colonies grow from founding to maturity, and across species, which are characterized by species-specific mature colony sizes. Second, eusocial colonies are tightly integrated, adaptive units whose members act cooperatively to maximize colony-level reproduction; they can therefore be viewed as 'individuals' (Hamilton et al. 2009), 'organisms' (Wheeler 1911; Queller and Strassmann 2009), or 'superorganisms' (Hölldobler and Wilson 2009), and they may be shaped by similar scaling relationships as functionally equivalent unitary organisms.

Scaling of division of labor

How might division of labor relate to colony size? In addition to the emergence hypotheses describe above, ultimate hypotheses also suggest that division of labor should scale positively with colony size in eusocial insects, due to functional costs in small colonies and/or efficiency benefits in larger colonies. Division of labor may be costly in small colonies if the need for specific tasks to be performed is too small and/or variable to keep specialists employed (Bell and Mooers 1997; Karsai and Wenzel 1998), or if the failure of a specialist disrupts an entire sequence of tasks (Oster and Wilson 1978; Herbers 1981). Alternatively, if increased colony size imposes physical and/or ecological constraints, then increased division of labor may be required to maintain colony efficiency (Bonner 2004).

Despite the confluence of emergence and functional hypotheses, which predict that division of labor should increase with colony size, empirical support is scarce and mostly indirect. Across species of wasps and ants, colony size is positively correlated with the degree of task specialization (Karsai and Wenzel 1998; Jeanne 2003) or worker polymorphism, which roughly approximates division of labor (Anderson and McShea 2001; Bonner 2004; but see Fjerdingstad and Crozier 2006). There is some evidence that task specialization increases with colony size within social insect species (Jeanne 1986; Gordon 1989; Thomas and Elgar 2003; but see Dornhaus et al. 2009), but those studies did not distinguish between effects of colony size and colony age, quantify ontogenetic changes within colonies, or manipulate colony size. The latter is required to determine whether scaling patterns emerge from short-term behavioral responses or are products of longer-term developmental processes.

***Pogonomyrmex*: a model for social scaling**

Chapters 4 and 5 examine the scaling of division of labor in the harvester ant *Pogonomyrmex californicus* (Formicidae: Myrmicinae). *Pogonomyrmex* *californicus* inhabits deserts and grasslands of the southwestern United States and northwestern Mexico; its colonies are founded by one or more queens and grow to contain up to several thousand workers at maturity (Johnson 2000a, 2004). Like most ants, and all bees and wasps, *P. californicus* workers are monomorphic (i.e., there are no distinct morphological subcastes). In Chapter 4, we test for colony-size effects on division of labor in undisturbed colonies, both during early colony growth and across same-aged colonies that vary naturally in size. In Chapter 5, we manipulate colony size and measure short-term individual and colony responses. To more fully explain how colony size influences the organization of work, we also analyze size-related changes in the proportional allocation of workers to tasks, worker activity, and the rate of contact between nestmates.

Broader significance

In sum, this dissertation presents original and rigorous empirical tests of leading theoretical models of the emergence and scaling of division of labor in insect societies. Four decades ago, with characteristic acumen, Wilson forecasted that "the reconstruction of mass behavior from a knowledge of the behavior of single colony members is the central problem of insect sociology" (1971, 227). Today, thanks to contributions from complexity science and new computational techniques, mathematical models of collective organization are proliferating at a faster pace than relevant empirical data are being collected (Beshers and Fewell 2001; Franks et al. 2009). We address this need by utilizing two tractable experimental systems, representing relatively simple and complex societies, to illuminate the dynamics of task organization as groups form, grow, and evolve. We hope that our work will inform and inspire further theoretical developments, and ultimately promote a better understanding of social behavior and complexity in insects and other taxa.

CHAPTER 2

EMERGENCE AND CONSEQUENCES OF DIVISION OF LABOR IN ASSOCIATIONS OF NORMALLY SOLITARY SWEAT BEES

Abstract: Division of labor is a pervasive feature of animal societies, but little is known about the causes or consequences of division of labor in non-eusocial cooperative groups. We tested whether division of labor self-organizes in an incipient social system: artificially induced nesting associations of the normally solitary sweat bee *Lasioglossum* (*Ctenonomia*) NDA-1 (Hymenoptera: Halictidae). We quantified task performance and construction output by females nesting either alone or with a conspecific. Within pairs, a division of labor repeatedly arose in which one individual specialized on excavation and pushing/tamping while her nestmate guarded the nest entrance. Task specialization could not be attributed to variation in overall activity, and the degree of behavioral differentiation was greater than would be expected due to random variation, indicating that division of labor was an emergent phenomenon generated in part by social dynamics. Excavation specialists did not incur a survival cost, in contrast to previous findings for ant foundress associations. Paired individuals performed more per capita guarding, and pairs collectively excavated deeper nests than single bees – potential early advantages of social nesting in halictine bees.

Introduction

Each of the major evolutionary transitions in biological organization, including multiple independent origins of animal societies, has been characterized by division of labor (Maynard Smith and Szathmáry 1995). In social systems, division of labor is a group-level pattern whereby different individuals specialize on different tasks (Michener 1974). Division of labor has been studied intensively in eusocial insects, for both reproductive and non-reproductive functions (Wilson 1971; Oster and Wilson 1978; Seeley 1982; Hölldobler and Wilson 1990, 2009; Robinson 1992; Beshers and Fewell 2001). However, the causes and consequences of division of labor in non-eusocial cooperative groups, which occur broadly across taxa, remain largely unknown (Bednarz 1988; Stander 1992; Underwood and Shapiro 1999; Costa and Ross 2003; Gazda et al. 2005; Arnold et al. 2005; Jeanson et al. 2005; Costa 2006). Recent theory suggests that division of labor can self-organize from local interactions among group members and their environment that generate behavioral differentiation, and may thus emerge spontaneously at the origins of sociality before becoming a target of natural selection (Page 1997; Fewell and Page 1999; Beshers and Fewell 2001; Camazine et al. 2001). We empirically tested whether division of labor is an emergent property of group living in artificially induced nesting associations of normally solitary sweat bees.

A prominent hypothesis for the self-organization of division of labor is the response threshold model, which postulates that an individual performs a given task when the external stimulus level it encounters exceeds an intrinsic response threshold (Robinson and Page 1989; Bonabeau et al. 1996, 1998; Page and Mitchell 1998; Beshers and Fewell 2001). When an individual with a low threshold for a task performs it, the stimulus for that task may decline, reducing the probability that others in the group will also perform it. That individual becomes the task specialist, and if different individuals perform different tasks, division of labor emerges. According to the general variance-based model, this feedback between task performance and stimulus intensity can amplify initially slight differences among individuals. Response thresholds may also be self-reinforced by experience: individuals who perform a task are more likely to continue performing it, and vice versa (Deneubourg et al. 1987; Plowright and Plowright 1988; Theraulaz et al. 1998). Alternatively, division of labor can be produced via behavioral dominance, if more dominant individuals force others to perform tasks (Hogeweg and Hesper 1983), and/or by spatial dynamics (Franks and Tofts 1994; Jeanson et al. 2005). These models, which are not mutually exclusive, have largely been developed for, and supported by, eusocial insects (e.g., Detrain and Pasteels 1991; Robinson 1992; O'Donnell 1998; Fewell and Bertram 1999; Powell and Tschinkel 1999; Fewell and Page 2000; O'Donnell 2001; O'Donnell and Foster 2001; Jones et al. 2004). However, they can also be applied to simpler, non-eusocial groups, and the same mechanisms could lead to the emergence of basic forms of division of labor early in social evolution (Page 1997; Fewell and Page 1999; Costa and Ross 2003; Helms Cahan and Fewell 2004; Jeanson et al. 2005; Jeanson et al. 2008; Jeanson and Fewell 2008).

Task specialization and division of labor have been observed in forced associations of normally solitary animals, including *Ceratina* carpenter bees (Sakagami and Maeta 1987), *Lasioglossum* sweat bees (Jeanson et al. 2005; Jeanson et al. 2008), and *Pogonomyrmex* seed-harvester ant queens that typically initiate colonies alone (Fewell and Page 1999; Helms Cahan and Fewell 2004; Jeanson and Fewell 2008). Evidence suggests that behavioral differentiation in these incipient groups can be achieved through a combination of response threshold variation and spatial dynamics (Fewell and Page 1999; Helms Cahan and Fewell 2004; Jeanson et al. 2005). Unlike previous studies, however, we quantified the performance of multiple tasks in concurrent solitary and social conditions; this design allowed task specialization to be dissociated from variation in overall activity and provided a controlled test for the emergence of division of labor via underlying changes in individual behavior, without confounding effects of prior social experience.

Surprisingly, induced pairs of solitary *Lasioglossum* and *Pogonomyrmex* species have repeatedly exhibited higher levels of task specialization and division of labor than communal or quasisocial congeners in which small groups of unrelated, reproductively active females nest together (Fewell and Page 1999; Helms Cahan and Fewell 2004; Jeanson et al. 2005; but see Jeanson and Fewell 2008). Division of labor can generate fitness disparities within *Pogonomyrmex* foundress associations; excavation specialists are more likely to die than non-specialists (Fewell and Page 1999; Helms Cahan and Fewell 2004), possibly reflecting increased energy expenditure and/or desiccation due to cuticular abrasion (Johnson 2000b). The maintenance of stable, non-kin, cooperative groups may thus require the sharing of costly tasks, equalizing fitness but reducing division of labor (Fewell and Page 1999; Helms Cahan and Fewell 2004). Here, we asked whether differentiation for excavation performance in incipient *Lasioglossum* societies could also have survival consequences that in turn shape the evolution of division of labor. In ground-nesting bees and wasps, excavation may impose costs in time, energy, body wear, and water loss, potentially favoring both social nesting and task generalization (Abrams and Eickwort 1981; Evans and Hook 1986; McCorquodale 1989; Danforth 1991).

We examined the emergence and consequences of division of labor during nest construction in artificially established associations of the normally solitary halictine bee *Lasioglossum* (*Ctenonomia*) NDA-1 (Hymenoptera: Halictidae). *Lasioglossum* is a cosmopolitan genus of ground-dwelling sweat bees that includes solitary, communal, and eusocial species, making it an ideal system for studying changes in division of labor during social evolution (Michener 1974; Wcislo 1997a; Schwarz et al. 2007). We measured task performance and nest construction output by bees nesting alone versus in forced pairs to determine (1) whether division of labor emerges in incipient groups, (2) how the transition from solitary to social nesting affects individual behavior and total construction output, and (3) whether excavation specialization reduces survival.

Methods

Collections

We collected adult females of *L.* NDA-1 in the Cobboboonee State Forest, southwestern Victoria, Australia (38.217°S, 141.558°E; 62 m elev) on 14, 16, and 18 Dec 2006. This population exhibits an annual, univoltine life cycle that begins in late spring, when overwintered, mated females construct new nests in exposed soil. Females are predominantly solitary; 17 of 19 excavated nests contained only one female, while two nests contained two females each (McConnell-Garner and Kukuk 1997). Low frequencies of two-female nests occur in many ‘solitary’ bees and wasps (Wcislo et al. 1993; Wcislo 1997a; Wcislo and Tierney 2009). Nests consist of vertical tunnels with lateral, wax-lined brood cells, which are mass provisioned with pollen and nectar.

We netted above nest aggregations between 10:00-13:00, when bees were foraging, and individually transferred each female to a vial kept in a cooler on ice. Upon return to the laboratory, each bee was marked on the thorax and abdomen with an enamel paint pen and held individually in a Petri dish provisioned with moistened filter paper and a 1:1 mixture of honey and water. Bees were maintained at ambient temperature overnight.

General activity

To determine whether differences in task performance during nest construction are associated with variation in general activity, we assayed individual activity levels and speed in circular, transparent plastic tubes (circumference = 20 cm, internal diameter = 4 mm) before introducing bees to nests (Breed et al. 1978; Jeanson et al. 2005). Trials were conducted 24 h after bees were collected, between 10:00-13:00 on day 1 of the experiment (15, 17, or 19 Dec, depending on date of collection), in homogeneously-lit arenas maintained at 27.0 ± 1.0°C. Each bee was individually loaded into a tube, allowed to acclimate for 2 min, and then observed for 5 min. We recorded amount of time spent active (walking forward or backward, or turning) versus inactive (immobile or grooming), and calculated speed by timing bees as they moved unidirectionally through an arc length of 10 cm. We obtained up to three speed measurements per individual when possible. Immediately following each activity trial, the test bee was returned to her individual Petri dish. Each section of tubing was used for only one trial per day and was washed with ethanol and water between days to remove any persistent odors.

Task performance during nest construction

At 18:00 on day 1, 5 h after the conclusion of activity trials, we introduced bees into vertical observation nests (12 cm wide × 15 cm deep × 3 mm thick) filled with moistened, sifted soil from the bees' nesting area. Each nest had a central, upper entrance connected by a 5-cm long plastic tube to a glass vial (diameter = 2 cm, height = 4 cm) provisioned with a 1:1 honey water solution. A vertical indentation of 1 cm was formed in the soil directly below the entrance to encourage the construction of a single tunnel. Nests were maintained at 30.0 ±

2.0°C under a photoperiod of 15 h light to 9 h dark (beginning at 19:00). We watered nests and replenished honey water *ad libitum*.

We randomly assigned individuals to one of two social contexts: ‘single’, in which bees nested alone (*n* = 39 individuals), or ‘paired’, in which nests were cohabited by two conspecifics (*n* = 74 individuals; 37 pairs). Members of each pair were introduced into nests simultaneously. We did not account for potential differences in age or body wear when pairing bees, but variation was assumed to be minimal because all individuals belonged to the same generation and were collected at the same time relatively early in the season, soon after nests were established. Behavior was sampled through a total of 90 scan surveys per individual distributed as follows: 30 scans from 19:00-21:30 on day 1, 30 scans from 8:00-10:30 on day 2, and 10 scans each from 16:00-16:50 on days 3, 4, and 5. Scans were performed every five min within each observation period. We sampled most intensively during the first 24 h because preliminary observations indicated that excavation declines thereafter, but we continued to observe behavior through day 5 to quantify temporal changes in activity. For each bee, we recorded performances of the following tasks:

Excavating: excavating soil with mandibles at distal end of tunnel.

Pushing: pushing loose soil with legs toward nest entrance or into vial.

Tamping: packing loose soil with abdomen into tunnel side walls.

Guarding: sitting in tube connecting nest and vial.

Foraging: extending proboscis into honey water solution in vial.

Bees that were not performing one of these specific tasks were classified as walking, self-grooming, or inactive (i.e., idle). Guarding is widely recognized in halictine bees as an individual sitting just inside the nest entrance and responding aggressively toward intruders (Michener 1974). Although natural enemies were absent from the laboratory, guards blocked the nest entrance with a defensive C-posture when disturbed by a toothpick.

Nest construction output

To estimate the amount of excavated soil, we digitally photographed nests following each observation period. Tunnel length and area were measured using Image J (National Institutes of Health: http://rsb.info.nih.gov/ij). We considered tunnel area to be directly proportional to tunnel volume, because all tunnels were as thick as the nests (3 mm).

Survival

We monitored individual survival daily through day 17. Dead bees were removed immediately upon detection.

Data analysis

Because individual performance can covary across tasks, we initially performed a repeated-measures MANOVA to test for overall effects of social context (single vs. paired) and time (days 1-5) on four response variables combined: per capita proportion of observations spent excavating, pushing/tamping (which generally co-occurred in space and time), guarding, and inactive. Foraging was not analyzed due to infrequent occurrence (< 1% of observations). Here, we used proportion of observations because the total number of scan samples per day decreased from 30/day on days 1-2 to 10/day on days 3-5. We treated each nest as an experimental unit and analyzed per capita task performance and inactivity (averaged within pairs) to statistically control for non-independence between nestmates. When Wilks' lambda values for overall effects on the combined response variables were significant ($P < 0.05$), we performed separate univariate repeated-measures ANOVA's on each response variable (Zar 1999). For within-subject effects (time and time×social context), we employed the Greenhouse-Geisser adjustment for deviations from the assumption of sphericity; corrected *P*-values are reported (Geisser and Greenhouse 1958; von Ende 1993). Two single bees and three pairs were excluded because an individual failed to perform any tasks throughout the experiment. Nests in which a bee died before day 5 were excluded from subsequent behavioral analyses; thus, sample sizes declined from 37 single individuals and 34 pairs on day 1 to 31 single individuals and 18 pairs on day 5.

Division of labor within each pair was quantified following the derivation of Shannon's diversity index (1948) by Gorelick et al. (2004; see for details). Calculations were based on the number of times each individual was observed excavating, pushing/tamping, or guarding. $\mathrm{DOL}_{\text{tasks-indiv}}$ (division of tasks across individuals) indicates the degree to which each task is performed by a subset of group members and ranges from 0 (no division of labor) to 1 (complete division of labor) when there at least as many tasks as individuals. Because we were interested in the expression of division of labor when there was an opportunity for it to occur (i.e., multiple individuals performing multiple tasks), we excluded pairs in which one individual failed to perform any tasks or both nestmates performed only one of the focal tasks. We did not calculate the complementary index $DOL_{indiv-tasks}$ (division of individuals into tasks) because individual specialization is restricted when there are more tasks than individuals, as in this study (Gorelick et al. 2004).

To further analyze behavioral differentiation and its survival consequences, we identified specialization on a potentially costly task – excavation. We designated a higher-frequency excavator (HFE) and a lower-frequency excavator (LFE) within each pair based on the nestmates' relative frequency of performance across days 1-2. Four pairs were excluded: two in which an individual died before day 2, one in which both members performed the same frequency of excavation, and one in which neither bee was observed excavating. Because the behaviors of nestmates were not independent, we tested for differences in task performance and inactivity between HFE and LFE bees and between HFE/LFE and single individuals using paired and 2-sample *t*-tests, respectively, with a sequential Bonferroni correction (Rice 1989) applied to each set of comparisons (adjusted *P*-values are reported).

We used a Monte Carlo simulation resampling procedure to determine whether behavioral differentiation within pairs was greater than would be expected due to random variation alone (Sokal and Rohlf 1995; Spessa et al.

2000). First, we calculated the mean ratio of observed excavation frequency between paired individuals (LFE/HFE, plus one pair with a ratio of 1; $n = 31$ pairs). Next, 10,000 iterations of 31 pairs each were sampled randomly with replacement from the single bees ($n = 37$), and the mean excavation frequency ratios (LFE/HFE as above) were used to generate a null distribution. We then calculated the proportion of expected excavation ratios that were lower (i.e., higher differentiation) than the observed ratio.

We conducted log-rank tests for effects of social context and excavation specialization on individual survival distributions across 17 days. Two single bees and two pairs were excluded because they escaped from their nests before the end of the experiment.

Data were arcsine- or ln-transformed to achieve normality and/or equal variance when necessary. All statistical tests were two-tailed and performed with STATISTICA (StatSoft, Inc.). Unspecified values are mean ± 1 SE.

Results

Effects of social context and time

Social context had a significant overall effect on per capita task performance and inactivity combined (rmMANOVA: $F_{4,44} = 4.35$, $P = 0.005$; Fig. 2.1). Subsequent univariate analyses revealed a significant difference only in guarding, which paired individuals performed approximately twice as frequently as single bees across days 1-5 (Table 2.1). Excavation, pushing/tamping, and inactivity did not differ between bees nesting alone versus in pairs.

Individual behavior also changed over time (rmMANOVA: $F_{16,32} = 6.74$, $P < 0.0001$; Fig. 2.1). Across days 1-5, there were declines in per capita excavation, pushing/tamping, and guarding (Table 2.1). Concordantly, bees were less active during days 3-5 than on days 1 and 2 (Tukey's pairwise comparisons: $P < 0.0001$). There were no significant interaction effects between social context and time (Table 2.1).

A preliminary comparison of tunnel length and tunnel area revealed that mean tunnel width was highly consistent across nests (2.9 ± 0.1 mm; $n = 40$); therefore, we used tunnel length to estimate nest construction output. Pairs dug nearly twice as far as single individuals over the course of the experiment (rmANOVA: $F_{1,47} = 6.65$, $P = 0.01$; cumulative tunnel length on day 5: single = 9.8 ± 1.4 cm, pairs = 17.5 ± 2.4 cm). Five pairs and two single bees excavated to the bottom of their nests between days 2-3, and a total of nine pairs and seven single bees reached the bottom by day 5, partly explaining the reduction in excavation and pushing/tamping over time.

Division of labor and task specialization

We calculated $\mathrm{DOL}_{\text{tasks-indiv}}$ – the degree to which each of the three focal tasks was performed by a single individual within each pair – using pooled task performance across days 1-2 only (60 scan surveys per individual), because bees reaching the bottom of their nests during days 3-5 experienced fewer available tasks. $\mathrm{DOL}_{\text{tasks-indiv}}$ averaged 0.25 ± 0.05 ($n = 31$ pairs).

We further analyzed task specialization by comparing higher-frequency versus lower-frequency excavators (HFE and LFE, respectively) within each pair. Designations were based on the relative frequency of excavation observed across days 1-2: HFE bees performed 79.4 ± 2.8% of the excavation within pairs (Fig. 2.2). To test whether asymmetry in excavation behavior was emergent, instead of reflecting general variation across the population, we performed a Monte Carlo simulation resampling procedure. The mean observed ratio of excavation performance between nestmates (0.33 ± 0.05) was lower than expected from random pairings of single bees ($P = 0.006$), indicating that social dynamics contributed to behavioral differentiation.

In addition, HFE bees also performed more pushing/tamping than LFE bees (HFE: 65.0 ± 6.1%; $t_{29} = 3.46$, Bonferroni-adjusted $P = 0.006$), but frequency of guarding did not differ significantly between nestmates (HFE: 42.8 ± 6.7%; $t_{29} = 1.38$, Bonferroni-adjusted $P = 0.2$; Fig. 2.2). Higher- and lower-frequency excavators exhibited similar overall activity in nests ($t_{29} = 1.11$, Bonferroni-adjusted $P = 0.5$; Fig. 2.2), pre-nesting circle tube activity ($t_{29} = 0.10$, $P = 0.9$), and speed within circle tubes ($t_{10} = 0.48$, $P = 0.6$).

Finally, we compared the task performance of HFE and LFE bees with that of bees nesting alone to learn how individual behavior changed in pairs (Fig. 2.2). Higher-frequency excavators and single bees did not differ in frequency of excavation ($t_{65} = 1.25$, Bonferroni-adjusted $P = 0.2$), pushing/tamping ($t_{65} = 0.006$, Bonferroni-adjusted $P = 0.996$), or guarding ($t_{65} = 1.63$, Bonferroni-adjusted $P = 0.2$). In contrast, LFE bees performed less excavation ($t_{65} = 2.55$, Bonferroni-adjusted $P = 0.03$) and pushing/tamping ($t_{65} = 0.238$, Bonferroni-adjusted $P = 0.04$), and more guarding ($t_{65} = 3.38$, Bonferroni-adjusted $P = 0.003$) than single bees. Inactivity during nest construction did not differ between single individuals and either HFE ($t_{65} = 2.10$, Bonferroni-adjusted $P = 0.12$) or LFE bees ($t_{65} = 0.88$, Bonferroni-adjusted $P = 0.4$), nor was it correlated with pre-nesting circle tube activity (Pearson correlation: $r_{101} = -0.12$, $P = 0.2$) or speed within circle tubes ($r_{59} = 0.05$, $P = 0.7$).

Survival

Individual survival was not affected by social context; 26 of 37 (70%) single bees and 44 of 70 (63%) paired bees survived through day 17 (log-rank test: $\chi^2 = 0.92$, $P = 0.4$). Moreover, probability of survival did not differ significantly between HFE and LFE bees; 22 of 28 (79%) HFE bees and 16 of 28 (57%) LFE bees survived (log-rank test: $\chi^2 = 1.71$, $P = 0.09$). In 12 pairs, only one member died – three were HFE and nine were LFE bees; this difference is also non-significant, although statistical power is limited (G test with Yates correction for continuity: $G = 2.15$, $P = 0.14$).

Discussion

Emergence of division of labor

When individuals of the normally solitary sweat bee *Lasioglossum* (*Ctenonomia*) NDA-1 were forced to establish nests with a conspecific, a division of labor repeatedly arose in which one bee specialized on nest construction tasks –

excavation and pushing/tamping – while her nestmate primarily guarded the nest entrance. Higher- and lower-frequency excavators differed in their allocation of performance across different tasks; specialization could not simply be attributed to variation in overall activity, as measured before and during nest construction. Moreover, the degree of behavioral differentiation between nestmates was greater than would be expected due to random variation in task performance among independent bees, indicating that division of labor was an emergent phenomenon generated in part by social dynamics. Our findings corroborate a growing body of evidence that basic forms of division of labor can self-organize in incipient groups of normally solitary animals that are not currently under selection for task specialization (Fewell and Page 1999; Helms Cahan and Fewell 2004; Jeanson et al. 2005; Jeanson et al. 2008; Jeanson and Fewell 2008).

In theory, behavioral differentiation could result from task specialists increasing their performance relative to a solitary state and/or non-specialists decreasing the frequency at which they perform a task in a social context. The later was true for nest construction in *L.* NDA-1; lower-frequency excavators performed less excavation and pushing/tamping than both higher-frequency excavators and single bees. A similar trend has been noted for excavation by *Pogonomyrmex* foundresses nesting alone versus in pairs (Fewell and Page 1999; Helms Cahan and Fewell 2004). This general pattern is consistent with the response threshold model: by decreasing the stimulus level associated with a given task, specialists reduce the probability that the task will be performed by other group members with higher thresholds (Robinson and Page 1989; Bonabeau et al. 1996; Beshers and Fewell 2001).

Spatial dynamics may have also contributed to the emergence of division of labor. Single and paired bees alike constructed nests typically consisting of a single, linear tunnel. Nestmates were thus prevented from excavating simultaneously, possibly channeling lower-frequency excavators toward the nest entrance, which they guarded more frequently than bees nesting alone. Moreover, the narrow confines of *Lasioglossum* nests require that individuals cooperatively rotate their bodies to pass one another (Breed et al. 1978; Kukuk 1992; McConnell-Garner and Kukuk 1997). Low tolerance to passing by *L.* NDA-1 (McConnell-Garner and Kukuk 1997) may restrict mobility throughout the nest, leading bees to encounter different tasks depending on their locations: excavation at the bottom of the nest, pushing/tamping along the length of a tunnel, and guarding at the nest entrance (Jeanson et al. 2005).

In some social insects, division of labor is driven by behavioral dominance, which itself can be an emergent property of group living (Hogendoorn and Schwarz 1998; Hogendoorn and Velthuis 1999; O'Donnell 1998, 2001; Powell and Tschinkel 1999; Hemelrijk 2002). However, we did not witness a single act of overt aggression (e.g., C-posture, biting, or lunging) between nestmates during many hours of observation. Furthermore, when social interactions of *L.* NDA-1 were previously assayed in circle tubes, there were no relationships among aggressive behavior, ovary condition, and/or subsequent task performance (McConnell-Garner and Kukuk 1997; Jeanson et al. 2005; Jeanson et al. 2008). These results contrast findings from forced associations of the solitary *Lasioglossum* (*Dialictus*) *figueresi*, in which bees with larger ovaries were more aggressive (Wcislo 1997b).

Survival consequences of specialization

Excavation specialization did not impose an individual survival cost, unlike in foundress associations of *Pogonomyrmex*, where greater mortality among excavation specialists may favor the evolution of task sharing (Fewell and Page 1999; Helms Cahan and Fewell 2004). Higher-frequency excavators actually tended to live longer than their nestmates, perhaps because individuals in poor condition were less likely to excavate, or because lower humidity near the nest entrance caused lower-frequency excavators to desiccate. In nature, within-group fitness disparities could be generated by differentiation for guarding and foraging, which are risky behaviors (Kukuk et al. 1998). The fitness consequences of these tasks, and how they are distributed across nestmates, are critical to understanding how selection shapes division of labor during early social evolution in halictine bees.

From solitary to social nesting

The artificial induction of social nesting by normally solitary bees caused spontaneous changes in task performance and nest construction output that could potentially provide selective advantages at the onset of group living, namely improved defense and shared construction costs. Paired individuals performed twice as much guarding as bees nesting alone, resulting in an approximate four-fold increase at the nest level. Guarding has been shown to protect brood from parasites and predators in other halictines, and is hypothesized to be a major benefit of group living in bees and wasps (Lin and Michener 1972; Eickwort et al. 1996; Kukuk et al. 1998; Smith et al. 2003; Wcislo and Tierney 2009). Social nesting relieves temporal constraints against guarding; one individual can guard the nest entrance while her nestmate(s) performs other tasks, including foraging. Morevover, alloparental guarding can provide assured fitness returns if a female dies before her mass-provisioned brood mature (Lin and Michener 1972; Gadagkar 1990; Queller 1993, 1994; Smith et al. 2003).

In addition, pairs collectively constructed deeper nests than single bees. Social nesting could thus reduce the individual costs of excavation (Evans and Hook 1986; McCorquodale 1989; Danforth 1991) and/or permit bees to reach soil levels with optimal water content (Abrams and Eickwort 1981). These effects may be especially important in arid regions with hard, compacted soils, such as the habitat of *L.* NDA-1.

Despite possible benefits of increased guarding and cooperative nest construction in multifemale associations, the focal population of *L.* NDA-1 is predominantly solitary. This paradox may be partly explained by avoidance of and/or aggression towards conspecifics. When compared to communal and eusocial *Lasioglossum* species, *L.* NDA-1 exhibits intermediate levels of aggression and relatively high frequencies of avoidance in circle tubes (McConnell-Garner and Kukuk 1997; Jeanson et al. 2005; see also Packer 2006).

Such behavior could preclude the initial formation of groups, even though aggressive interactions are rare or nonexistent when bees are forced to share nests. Furthermore, sociality may be associated with costs, including intraspecific brood parasitism and/or fitness disparities related to task specialization (Helms Cahan and Fewell 2004; Wcislo and Tierney 2009).

Table 2.1. Effects of social context (single vs. paired) and time (days 1-5) on per capita task performance and inactivity.

		Excavating		Pushing/Tamping		Guarding		Inactive	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Between-subjects									
Social context	1, 47	0.11	0.7	0.29	0.6	16.47	<0.001	1.14	0.3
Within-subjects									
Time	4, 188	10.21	<0.001	13.39	<0.001	8.22	<0.001	3.32	<0.001
Time×social context	4, 188	0.21	0.9	0.32	0.9	2.14	0.08	0.35	0.8

Response variables were proportions of total observations during which individuals performed tasks or were inactive. Each response variable was analyzed in a separate rmANOVA. Adjusted Greenhouse-Geisser *P* values are reported for within-subjects effects.

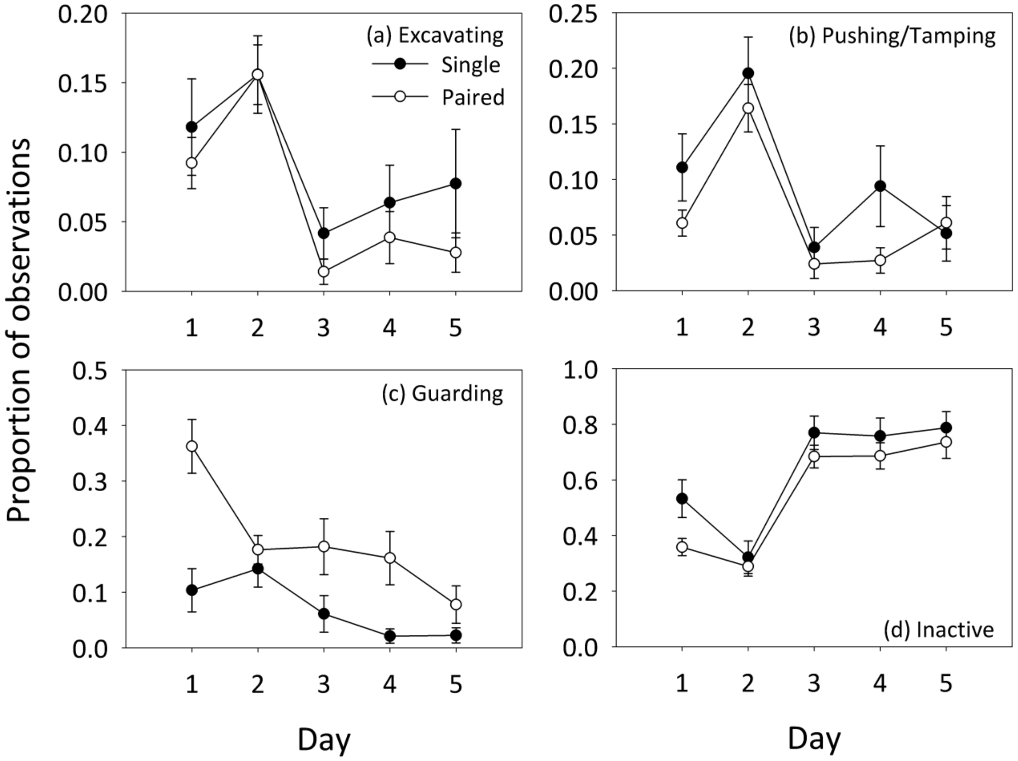

Figure 2.1. Per capita task performance by bees nesting alone vs. in artificially established pairs. Values are mean (± SE) proportion of observations per scan survey (30 surveys per bee per day on days 1-2, 10 surveys per bee per day on days 3-5). Due to mortality, sample sizes declined from 37 single individuals and 34 pairs on day 1 to 31 single individuals and 18 pairs on day 5.

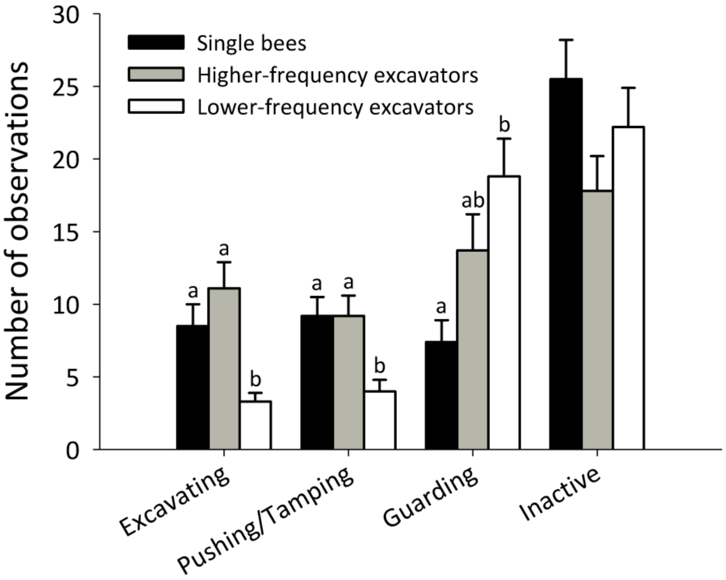

Figure 2.2. Behavioral differentiation within induced pairs, relative to bees nesting alone. Each pair contained a higher-frequency excavator (HFE) and a lower-frequency excavator (LFE), classified post-hoc using relative performance. Values are mean (± SE) number of observed task performances per individual across days 1-2 (30 scan surveys per day). For each task, different letters indicate statistical significance after sequential Bonferroni correction. $n_{\text{single}} = 37$, $n_{\text{HFE}} = 30$, $n_{\text{LFE}} = 30$.

CHAPTER 3

INCREASED GROUP SIZE PROMOTES HIGHER DIVISION OF LABOR IN *DE NOVO* GROUPS OF SWEAT BEES

Abstract: Division of labor is a fundamental attribute of animal societies. Self-organizational theory suggests that division of labor can emerge spontaneously at the origin of group living, and that increased group size further promotes task specialization. We examined the emergence and scaling of division of labor in an evolutionarily incipient social system: *de novo* communal groups of the normally solitary sweat bee *Lasioglossum* (*Ctenonomia*) NDA-1. A division of labor repeatedly arose between nest excavation and guarding in pairs and in groups of four bees. Moreover, division of labor was higher in larger groups. Thus, we show that increased division of labor can be an emergent consequence of increased group size, in the absence of direct selection for task specialization. Behavioral differentiation may be facilitated by spatial organization; individuals in larger groups were less mobile throughout the nest, possibly exposing them to fewer tasks. Although a basic division of labor emerged in newly-formed groups, its functionality was limited; to realize benefits of division of labor, groups must evolve mechanisms of coordination.

Introduction

The coalescence of individual organisms into cooperative groups may give rise to novel group-level phenotypes that provide substrates for further social evolution. One such phenotype is division of labor, broadly defined as a pattern whereby different individuals perform different functions, even if only temporarily (Michener 1974; Fewell et al. 2009). Division of labor is best known from eusocial insect colonies, in which it presumably enhances individual- and colony-level performance (Wilson 1971; Oster and Wilson 1978; Hölldobler and Wilson 1990, 2009). But it occurs to varying degrees across a wide range of taxa and social structures (Fetherston et al. 1990; Stander 1992; Lacey and Sherman 1997; Underwood and Shapiro 1999; Clutton-Brock et al. 2003; Arnold et al. 2005; Gazda et al. 2005; Ridley and Raihani 2008; Bruintjes and Taborsky 2010), and it may even be an emergent property of group living at the origins of sociality (Page 1997; Fewell and Page 1999; Jeanson et al. 2005; Holbrook et al. 2009).

Theory and empirical observations suggest that division of labor can self-organize from nonlinear interactions among group members and the local environment, without central control (reviewed by Beshers and Fewell 2001). Proposed mechanisms include the amplification of intrinsic differences in task propensity (response threshold model; Robinson and Page 1989; Bonabeau et al. 1998; Page and Mitchell 1998) and spatial segregation or sorting, which may be induced by agonistic interactions and can expose different individuals to different tasks (Tofts and Franks 1992; Powell and Tschinkel 1999; O'Donnell 2001).

Although self-organizational models of division of labor have been inspired largely by eusocial insects, their basic requirements may be present early in social evolution; thus, division of labor could be generated in newly-formed groups, preceding selection for task specialization (Page 1997; Fewell and Page 1999; Holbrook et al. 2009). Moreover, as groups increase in size, division of labor is predicted to increase automatically (Gautrais et al. 2002; Merkle and Middendorf 2004; Jeanson et al. 2007). To investigate the emergence and scaling of division of labor in an evolutionarily incipient social system, we created *de novo* 'communal' groups of the solitary sweat bee *Lasioglossum* (*Ctenonomia*) NDA-1.

Sweat bees (Halictidae), including the genus *Lasioglossum*, are ideal for studying the evolution of social organization, which is highly labile within and among species (Michener 1974; Wcislo 1997a; Schwarz et al. 2007; Wcislo and Tierney 2009). Females of some species exhibit predominantly solitary life-histories, whereas others are facultatively or obligately eusocial, with reproductive division of labor. Intermediate in size and complexity, but not representing a transitional stage (Kukuk 1992; Schwarz et al. 2007; Wcislo and Tierney 2009), are communal groups in which adult females share a nest, with each provisioning her own offspring. A powerful approach to examining the origins of social behaviors is to simulate the initial evolution of group living by forcing normally solitary bees to cohabit artificial nests (Sakagami and Maeta 1987; McConnell-Garner and Kukuk 1997; Wcislo 1997b; Jeanson et al. 2005). In forced pairs of *L.* NDA-1, a basic division of labor emerges between nest excavation and guarding; intrinsic behavioral variability and spatial segregation have been implicated as potential underlying factors (Jeanson et al. 2005; Holbrook et al. 2009). Naturally communal bees live in groups ranging from just two adults to several dozen (Michener 1974); changes in group size may be particularly important for the organization of work as the ratio of individuals-to-tasks surpasses one, increasing the opportunity for specialization. Here, we quantified task performance, spatial organization, and agonistic interactions in groups of up to four individuals, performing three major tasks during nest founding.

Methods

Collections

We collected adult females of *L.* NDA-1 in the Cobboboonee State Forest, southwestern Victoria, Australia (38.217°S, 141.558°E; 62 m elev) on 4, 6, and 10 Jan 2009. We netted above nest aggregations between 10:00-13:00 hrs, when bees were foraging. Individuals were kept in separate vials and chilled until later in the day, when each was marked on the thorax and abdomen with an enamel paint pen and transferred to a Petri dish provisioned with moistened filter paper and a 1:1 mixture of honey and water. Bees were maintained in the laboratory at ambient temperature overnight. The general protocol followed previous studies (Jeanson et al. 2005; Holbrook et al. 2009).

Day 1: Nesting alone

To test whether the task roles of group members result, in part, from intrinsic differences, we observed all bees nesting alone on Day 1, before some were assigned to groups on Day 2. At 07:00 hrs on the day following collection, we transferred each individual to a separate, glass observation nest (interior dimensions: 12 cm wide × 15 cm deep × 3 mm thick) filled with moistened, sifted soil from the collection site. The thickness of nests approximated the size of a bee. Each nest had a single entrance in the center of the upper margin, connected by a 5-cm long plastic tube to a glass vial (diameter = 2 cm, height = 4 cm) provisioned with a 1:1 honey water solution. We formed a 1-cm vertical indentation in the soil directly below the entrance to promote excavation. Once all nests were loaded (by 08:00 hrs), they were arranged upright in a well-lit room maintained at 30.0 ± 1.0°C.

One hr after bees were introduced to nests, we began behavioral observations. We performed 80 instantaneous samples per bee (Altmann 1974) over the next 15 hrs, with successive observations separated by at least 10 min. The following tasks were recorded:

Excavating: excavating soil with mandibles at distal end of tunnel.

Pushing: pushing loose soil with legs toward nest entrance or into vial.

Tamping: packing loose soil with abdomen into tunnel side walls.

Guarding: sitting in tube connecting nest and vial.

Foraging: extending proboscis into honey water solution in vial.

Other behaviors included self-grooming, walking, and flying in the vial. Individuals that were not performing a specific task or other behavior were classified as inactive. In halictine bees, ‘guarding’ is generally defined as sitting inside the nest entrance and responding aggressively toward intruders (Michener 1974). Natural enemies were absent from the laboratory, but when we inserted a toothpick into nests after the experiment, guards typically advanced and/or defensively C-postured. During each sample, we also noted whether the bee was located in: (1) the vial, (2) the tube, (3) the upper portion of a tunnel, or (4) the distal end of a tunnel.

After 15 hrs, we digitally photographed nests to measure the area of excavated soil (all tunnels were as thick as the observation nests). Bees were returned to their individual Petri dishes and maintained at ambient temperature overnight.

Day 2: Nesting alone, in pairs, or in groups of four

On Day 2, we repeated the procedure from Day 1, except that individuals were randomly assigned to one of three ‘social context’ treatment levels: (1) nesting alone (singleton), (2) nesting with one conspecific in a pair, (3) or nesting with three conspecifics in a group of four (quad). Within groups (pairs and quads), nestmates were randomly assigned and transferred simultaneously to their new nests. We sampled behavior as above, performing 80 scan samples during which the behavioral state and location of each individual, and whether she was physically contacting a nestmate, was noted at the instance when she was first seen. We also recorded all aggressive interactions (e.g., lunging, biting, and C-posturing) that occurred during a group scan. Nests were photographed after 15 hours to measure excavation output. After excluding nests in which an individual died or failed to perform any tasks (suggesting poor condition), we observed and analyzed 31 singletons, 28 pairs, and 28 quads (199 bees total across Days 1-2).

Data analysis

We quantified division of labor in pairs and quads using the $\mathrm{DOL}_{\text{indiv-tasks}}$ index, which represents the overall extent to which individuals specialize on a subset of tasks, and permits statistical comparison of groups of varying size (Gorelick et al. 2004; Jeanson et al. 2007; Dornhaus et al. 2009). For each group, we generated a normalized matrix of task performance using the number of times each individual was observed excavating, pushing/tamping, or guarding on Day 2. Pushing and tamping were combined because they co-occurred in space and time (following Jeanson et al. 2005; Holbrook et al. 2009). Foraging was excluded because it was rare. $\mathrm{DOL}_{\text{indiv-tasks}}$ was calculated as mutual entropy between individuals and tasks ($I_{\text{indiv,tasks}}$) divided by Shannon's index or marginal entropy of tasks (H_{tasks}). For details, see Gorelick et al. (2004), but note that the definitions of the indices $\mathrm{DOL}_{\text{indiv-tasks}}$ and $\mathrm{DOL}_{\text{tasks-indiv}}$ are switched (Dornhaus et al. 2009).

Division of labor is a group-level attribute whereby individuals perform a subset of tasks and tasks are performed by a subset of individuals (Gorelick et al. 2004). To further analyze underlying changes in individual-level task specialization, we also calculated Shannon's task diversity index for each individual:

$$H_{\text{tasks}} = -\sum_{i=1}^{s} p_i \log p_i \,,$$

where s = number of tasks performed and p_i = proportion of task performance allocated to the i^{th} task. An increase in H_{tasks} (i.e., a reduction in task specialization) can be caused by an increase in the number of tasks (s) and/or an increase in the evenness of performance across tasks, defined as

$$J_{\text{tasks}} = \frac{H_{\text{tasks}}}{H_{\max}},$$

where $H_{\max} = \log s$ (Pielou 1966). In addition, we calculated individual spatial diversity (H_{loc}), or the degree to which individuals moved throughout the nest, substituting the four nest locations for the three tasks in the expressions above. When calculating $\text{DOL}_{\text{indiv-tasks}}$ and diversity indices, we excluded eight pairs and two quads because one of the three tasks was not performed by any individual ($n_{\text{pairs}} = 20$, $n_{\text{quads}} = 26$).

Nest area was measured using ImageJ (National Institutes of Health: http://rsb.info.nih.gov/ij). We subtracted 0.293 cm^2 from each nest to account for the preexisting indentation.

Because some response variables were non-normally distributed, we used nonparametric tests throughout. For individual-level responses, we treated each nest as an experiment unit and analyzed per capita values in pairs and quads. Analyses were performed using STATISTICA (StatSoft, Inc.).

Results

Group size and division of labor

Quads exhibited higher division of labor ($\mathrm{DOL}_{\text{indiv-tasks}}$) than pairs (Mann-Whitney test: $U = 145$, $n_{\text{pairs}} = 20$, $n_{\text{quads}} = 26$, $P = 0.01$; $\text{median}_{\text{pairs}} = 0.13$; $\text{median}_{\text{quads}} = 0.35$; Fig. 3.1a). Average task diversity (H'_{tasks}), an individual-level measure of task generalization, was strongly, negatively correlated with $\mathrm{DOL}_{\text{indiv-tasks}}$ (Spearman rank correlation: pairs: $r_s = -0.75$, $P = 0.0001$, $n = 20$; quads: $r_s = -0.61$, $P = 0.001$, $n = 26$), and was correspondingly lower in quads than in pairs ($U = 102$, $P < 0.001$; $\text{median}_{\text{pairs}} = 0.33$, $\text{median}_{\text{quads}} = 0.19$). Contributing to the effect of group size on task diversity were significant differences in the number of tasks performed ($U = 152.5$, $P = 0.02$; $\text{median}_{\text{pairs}} = 2.75$, $\text{median}_{\text{quads}} = 2.25$; Fig. 3.1b) and in the evenness of task performance ($U = 120$, $P = 0.002$; $\text{median}_{\text{pairs}} = 0.82$, $\text{median}_{\text{quads}} = 0.59$; Fig. 3.1c).

To more closely inspect task differentiation within groups, we designated higher-frequency excavators (HFE's) as those individuals performing over 50% of the total excavation in pairs or over 25% in quads, and compared their performance of other tasks to that of their lower-frequency excavator (LFE) nestmates. In pairs, LFE's consistently performed more guarding than HFE's (Wilcoxon signed-rank test: $T_{20} = 17.5$, $P = 0.001$), but nestmates did not differ in frequency of pushing-tamping ($T_{18} = 43.5$, $P = 0.07$) or in overall activity ($T_{18} = 76.0$, $P = 0.17$; Fig. 3.2a). Differences were more pronounced in quads: HFE's performed more pushing-tamping ($T_{25} = 57.5$, $P = 0.005$), while LFE's guarded the nest more frequently ($T_{26} = 8.0$, $P < 0.0001$) and were more active overall ($T_{26} = 62.0$, $P = 0.004$; Fig. 3.2b). The number of HFE's per quad ranged from 1 to 3 (median and mode = 2).

Intrinsic differences underlying task roles

Unfortunately, most bees did not excavate when they nested alone on Day 1, preventing us from testing whether task roles in groups could be predicted from preexisting, intrinsic differences. The change in excavation between Days 1 and 2 was not an effect of social nesting; even among singletons, the proportion of individuals that excavated increased from only 45% on Day 1 to 74% on Day 2 (chi-squared test: $\chi^2_1 = 5.43$, $P = 0.02$).

Spatial organization

Spatial diversity (H_{loc}), or the degree to which individuals moved throughout the nest, was lower in quads than in pairs (Mann-Whitney test: $U = 155$, $P = 0.02$,; $\text{median}_{\text{pairs}} = 0.36$, $\text{median}_{\text{quads}} = 0.26$; Fig 3.3). Individuals in quads were observed in fewer of the four nest locations ($U = 150.0$, $P = 0.02$,

$\text{median}_{\text{pairs}}$ = 3.50, $\text{median}_{\text{quads}}$ = 3.25), but spatial evenness was not affected by group size (U = 210, P = 0.27, $\text{median}_{\text{pairs}}$ = 0.67, $\text{median}_{\text{quads}}$ = 0.61).

Social interactions

Nestmates came into contact with one another more frequently, per capita, in quads than in pairs (Mann-Whitney test: U = 68, n_{pairs} = 28, n_{quads} = 28, $P <$ 0.0001; $\text{median}_{\text{pairs}}$ = 1 instance out of 80 samples, $\text{median}_{\text{quads}}$= 11 out of 80). Aggressive interactions were rarely observed (42 occurrences out of 4,480 group scans), but were more likely to occur in quads than in pairs (12 vs. 2 groups; chi-squared test: χ^2_1 = 17.11, P < 0.0001). Among quads, individuals were more mobile throughout the nest when aggression was observed (H_{loc}; U = 23, n_{present} = 12, n_{absent} = 14, P = 0.002; $\text{median}_{\text{present}}$ = 0.36, $\text{median}_{\text{absent}}$ = 0.24), but there was no association between the occurrence of aggression and $\text{DOL}_{\text{indiv-tasks}}$ (U = 49, n_{present} = 12, n_{absent} = 14, P = 0.08; $\text{median}_{\text{present}}$ = 0.26, $\text{median}_{\text{absent}}$ = 0.40). Higher- and lower-frequency excavators did not differ in their likelihood of delivering or receiving aggression (chi-squared tests: delivery: χ^2_1 = 0.01, P = 0.92; reception: χ^2_1 = 0.36, P = 0.55).

Effects of social nesting on individual and nest-level task performance

Across the three nesting conditions (singletons, pairs, quads), there were no differences in per capita frequency of excavation (Kruskal-Wallis test: H_2 = 0.31, n_{singles} = 31, n_{pairs} = 28, n_{quads} = 28, P = 0.86) or pushing-tamping (H_2 = 0.20,

$P = 0.90$; Fig. 3.4). However, individuals in groups (pairs and quads) performed more guarding ($H_2 = 28.29$, $P < 0.0001$; pairwise comparisons of mean ranks: $P < 0.001$) and were more active ($H_2 = 12.45$, $P = 0.002$; pairwise comparisons: $P \leq 0.01$) than singleton bees. Individuals in pairs and quads did not differ in guarding or overall activity (pairwise comparisons: $P \geq 0.64$).

Nest size was highly variable, but was larger in quads than in singletons ($H_2 = 11.22$, $P = 0.004$; pairwise comparison: $P = 0.003$; Fig. 3.5a); nest area did not differ significantly between singletons and pairs or between pairs and quads ($P \geq 0.10$; $\text{median}_{\text{singles}} = 0.2\ \text{cm}^2$, $\text{median}_{\text{pairs}} = 0.4\ \text{cm}^2$, $\text{median}_{\text{quads}} = 1.1\ \text{cm}^2$). Finally, nest-level frequency of guarding, or the number of scan samples during which the nest entrance was guarded by any individual, was higher in larger groups ($H_2 = 55.07$, $P < 0.0001$; pairwise comparisons: $P < 0.004$; $\text{median}_{\text{singles}} =$ 2 out of 80 scans, $\text{median}_{\text{pairs}} = 35$, $\text{median}_{\text{quads}} = 72$; Fig. 3.5b).

Discussion

Emergence and scaling of division of labor

When females of the normally solitary *L.* NDA-1 are experimentally coerced into 'communal' groups, a division of labor repeatedly arises between excavation and nest guarding tasks. This observation further supports the notion that division of labor can emerge during early social evolution, providing a novel substrate for subsequent selection (Fewell and Page 1999; Helms Cahan and Fewell 2004; Jeanson et al. 2005; Jeanson and Fewell 2008; Jeanson et al. 2008;

Holbrook et al. 2009). Moreover, when group size is experimentally increased from two to four adults, individuals become more specialized and division of labor increases. The positive scaling of division of labor with group size is consistent with predictions of self-organizational theory (Gautrais et al. 2002; Merkle and Middendorf 2004; Jeanson et al. 2007). Task specialization also increases with colony size among larger and more integrated eusocial insect colonies, which may adaptively regulate division of labor in response to changes in size (Oster and Wilson 1978; Jeanne 1986; Thomas and Elgar 2003; Bonner 2004; Holbrook et al. in press). However, this study shows that functional integration and colony-level selection are not required to produce a scaling relationship; increased group size spontaneously promotes higher division of labor in a *de novo* social system (see also Jeanson and Fewell 2008). Our findings are most relevant to the evolution of communal nesting, which represents an alternative social trajectory to eusociality in sweat bees (Kukuk 1992; Schwarz et al. 2007; Wcislo and Tierney 2009). Increases in the size of these relatively small groups (Michener 1974) may drastically alter their organization, particularly as the number of individuals exceeds the number of tasks, thereby increasing the opportunity for task specialization.

Spatial organization

Spatial organization may contribute to the emergence and scaling of division of labor in *L.* NDA-1. Individuals in larger groups exhibit lower spatial diversity; i.e., they move between fewer regions of the nest. Because tasks are unevenly distributed in space, reduced mobility may expose individuals to fewer tasks, leading to increased specialization (Tofts and Franks 1992; Sendova-Franks and Franks 1995; Robinson et al. 2009). In other insect societies, spatial sorting or segregation of individuals is induced by agonistic interactions (Powell and Tschinkel 1999; Robson et al. 2000; O'Donnell 2001). The narrow diameter of *Lasioglossum* nests requires nestmates to cooperatively rotate their bodies to pass one another; females of *L.* NDA-1are relatively intolerant of passing, which likely restricts movement through the nest (McConnell-Garner and Kukuk 1997; Jeanson et al. 2005). Overt acts of aggression, however, were rare, and individuals were more mobile in groups with aggressive interactions, suggesting that they are not sorted via aggression *per se*.

Functional consequences of social nesting and division of labor

A likely benefit of social nesting in bees is improved defense against predators and parasites through nest guarding (Lin and Michener 1972; Abrams and Eickwort 1981; Kukuk et al. 1998; Wcislo and Tierney 2009). In our experiment, the frequency of guarding increased dramatically with group size; on average, quads’ nests were guarded approximately 90% of the time, compared to less than 50% in pairs and less than 10% in singletons. The presence of multiple group members increases the probability that at least one will be available to guard the nest entrance while her nestmates engage in other activities. Therefore, nests of larger groups may automatically be better protected than those of smaller groups or solitary individuals.

Might division of labor confer functional advantages to evolutionarily incipient groups, as hypothesized for more complex and derived societies (Oster and Wilson 1978; Dornhaus 2008; Chittka and Muller 2009)? Division of labor can be viewed as a structural phenotype; its functionality in task allocation, however, depends on whether it is accompanied by mechanisms of group-level coordination, or 'social physiology' (Seeley 1995; Johnson and Linksvayer 2010). Unlike eusocial colony members, who respond to myriad social cues and signals (Wilson and Hölldobler 1988; Hölldobler and Wilson 1990, 2009; Seeley 1995), individuals in forced associations of *L.* NDA-1 appear to perform tasks with little, if any, reference to their nestmates; sometimes, they even interfere with one another's work, such as by filling in a tunnel that was previously excavated. Thus, while rudimentary division of labor can arise spontaneously at the genesis of group living, its fitness consequences and evolutionary fate are contingent upon the evolution of communication and coordination between group members.

Figure 3.1. Division of labor, per capita number of tasks, and evenness of task performance in pairs vs. quads of *L.* NDA-1. $\mathrm{DOL}_{\text{indiv-tasks}}$ represents the degree to which individuals specialize on subsets of tasks. Boxes show median and 1st and 3rd quartiles; whiskers show non-outlier range (± 1.5 interquartile range). $n_{\text{pairs}} = 20$, $n_{\text{quads}} = 26$.

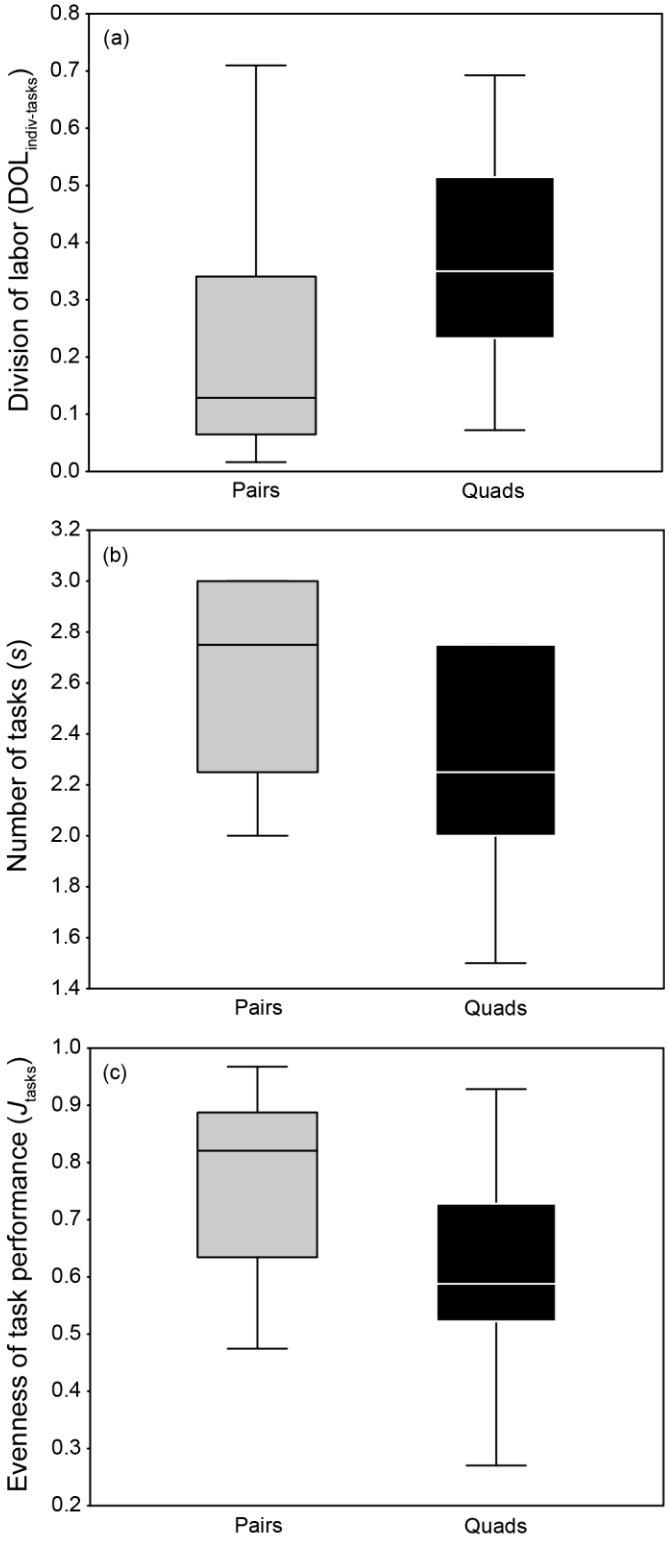

Figure 3.1, continued.

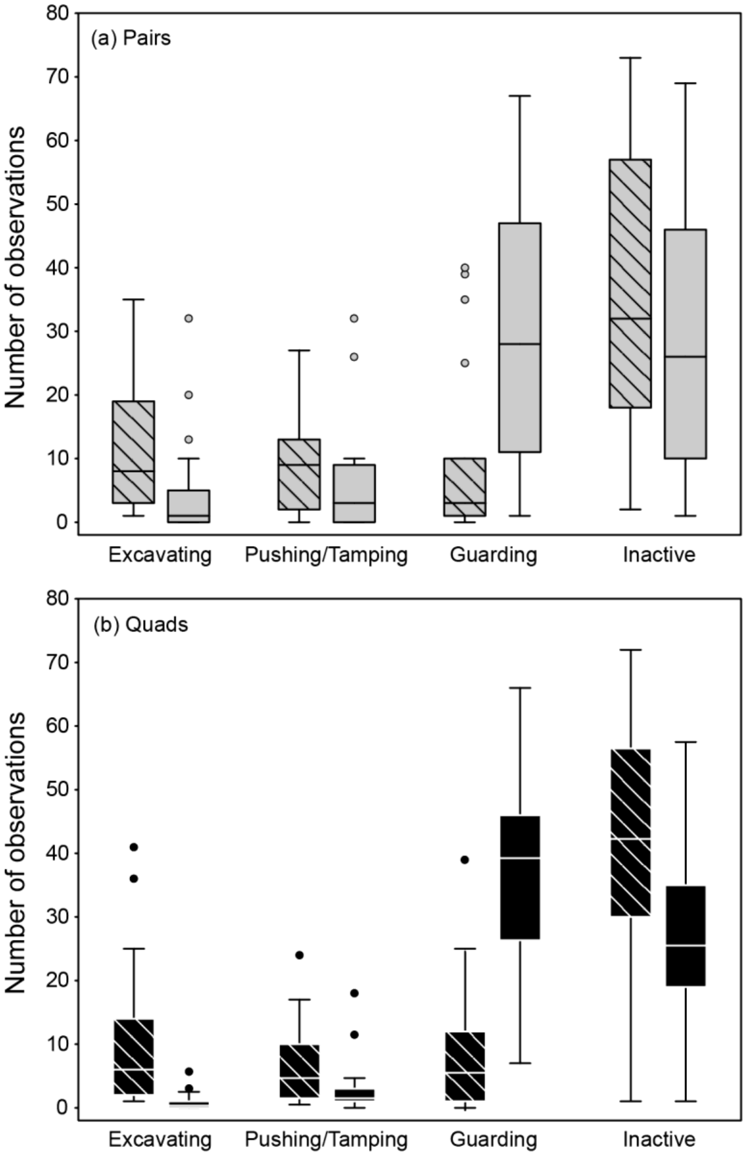

Figure 3.2. Task performance by higher-frequency excavators (diagonal hatching) vs. lower-frequency excavators (solid boxes) within (a) pairs and (b) quads of *L.* NDA-1. Number of observations is per capita frequency out of 80 samples per individual. Boxes show median and 1st and 3rd quartiles; whiskers show non-outlier range (± 1.5 interquartile range); circles are outliers. $n_{\text{pairs}} = 20$, $n_{\text{quads}} = 26$.

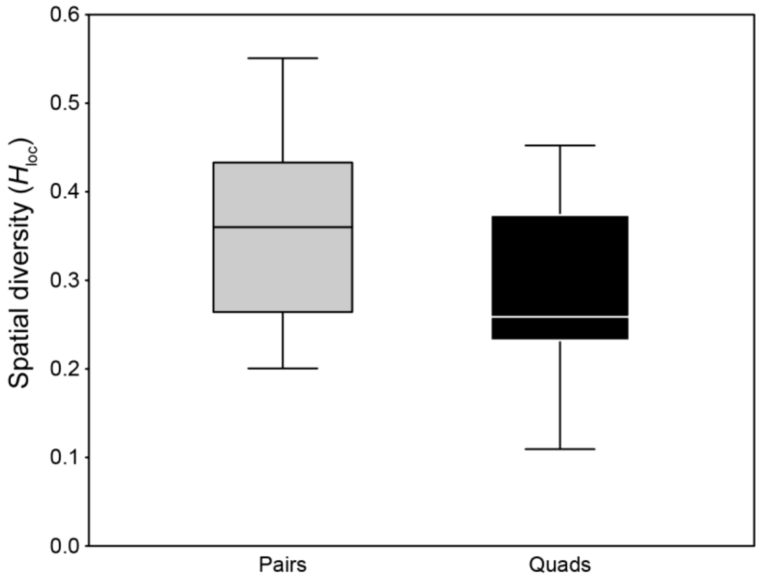

Figure 3.3. Individual spatial diversity in pairs vs. quads of *L*. NDA-1. H_{loc} represents the degree to which individuals move throughout the nest. Boxes show median and 1st and 3rd quartiles; whiskers show non-outlier range (± 1.5 interquartile range). $n_{\mathrm{pairs}} = 20$, $n_{\mathrm{quads}} = 26$.

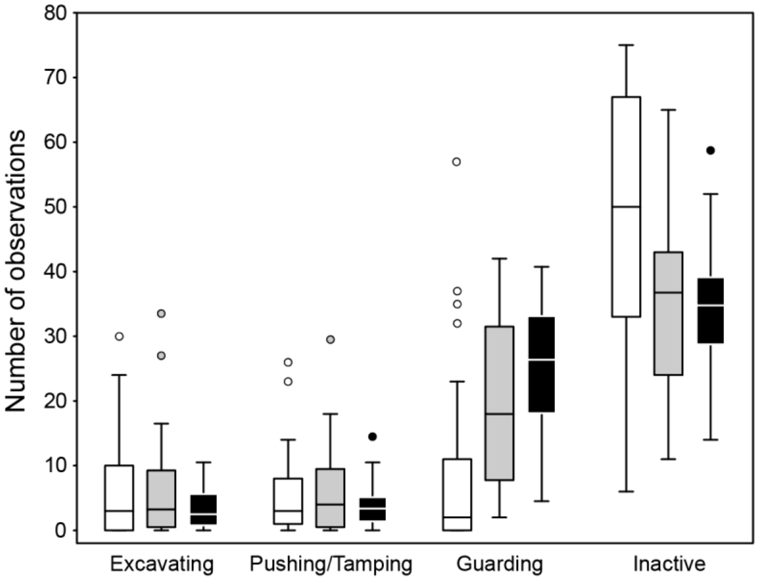

Figure 3.4. Per capita task performance by bees nesting alone (white), in pairs (gray), or in quads (black). Number of observations is per capita frequency out of 80 samples per individual. Boxes show median and 1st and 3rd quartiles; whiskers show non-outlier range (± 1.5 interquartile range); circles are outliers. n_{singles} = 31, n_{pairs} = 28, n_{quads} = 28.

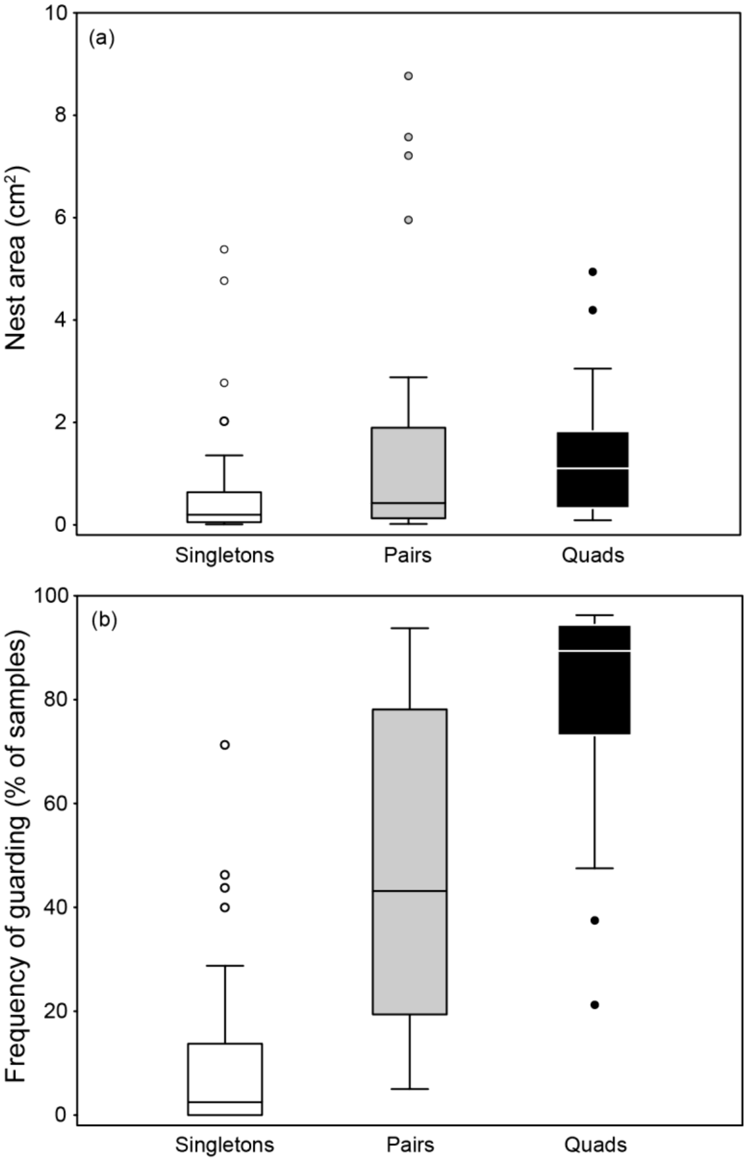

Figure 3.5. Excavated nest size and frequency of guarding in singletons, pairs, and quads of *L.* NDA-1. Frequency of guarding is percentage of scan samples during which the nest entrance was guarded by any individual. Boxes show median and 1st and 3rd quartiles; whiskers show non-outlier range (± 1.5 interquartile range); circles are outliers. $n_{\text{singles}} = 31$, $n_{\text{pairs}} = 28$, $n_{\text{quads}} = 28$.

CHAPTER 4

DIVISION OF LABOR INCREASES WITH COLONY SIZE IN THE HARVESTER ANT *POGONOMYRMEX CALIFORNICUS*

Abstract: Size has profound consequences for the structure and function of biological systems, across levels of organization from cells to social groups. As tightly integrated units that vary greatly in size, eusocial insect colonies, in particular, are expected to exhibit social scaling relations. To address the question of how social organization scales with colony size, we quantified task performance in variably sized colonies of the harvester ant *Pogonomyrmex californicus*. We found a positive scaling relationship between colony size and division of labor, in two different contexts. First, individual workers were more specialized in older, larger colonies. Second, division of labor increased with colony size, independently of colony age. Moreover, the proportional allocation of workers to tasks shifted during colony ontogeny – older, larger colonies performed relatively less brood care – but did not vary with colony size among same-aged colonies. There were no colony-size effects on per capita activity or the distribution of activity across workers. Size-related changes in task performance were correlated with changes in the rate of encounter between nestmates. These results highlight the importance of colony size for the organization of work in insect societies, and raise broader questions about the role of size in sociobiology.

Introduction

Biology is largely a matter of size. Body size has profound consequences for the structure, physiology, behavior, ecology, and life history of organisms; these relations are described by biological scaling ‘rules’ or ‘laws’ whose underlying mechanisms can reveal general principles of life (reviewed by Peters 1983; Schmidt-Nielsen 1984; Brown and West 2000; Dial et al. 2008). Just as the size of a multicellular body is determined by the number and size of cells it contains, the size of a social group or colony can be defined by the number and size of individual members it comprises. By extension, the form and function of groups may be shaped by *social scaling* relations, or predictable changes in individual- and group-level properties in response to changes in group size (Jun et al. 2003; Bonner 2004; Yip et al. 2008).

Social scaling patterns, sometimes referred to as ‘group-size effects’, have been recognized in diverse taxa, for traits ranging from metabolic rate to vigilance (Elgar 1989; Barton 1996; Nakaya et al. 2003; Yip et al. 2008; Pollard and Blumstein 2008, 2011). Colony size appears to play a particularly important role in the structure and organization of eusocial insect colonies, such as those of ants, termites, and some bees and wasps (Tschinkel 1993; Bourke 1999; Anderson and McShea 2001; Jeanne 2003; Hou et al. 2010; Waters et al. 2010). Two features of social insects may increase their susceptibility to social scaling. First, they exhibit tremendous variation in colony size, from fewer than 10 individuals to tens of millions; colonies grow during ontogeny, and mature colony size varies within and among populations and species (Wilson 1971; Michener 1974; Hölldobler and Wilson 1990). Second, eusocial colonies of all sizes are tightly integrated, adaptive units that are, in many ways, functionally equivalent to unitary organisms, and may thus experience analogous scaling effects (Wheeler 1911; Wilson 1985; Hölldobler and Wilson 1990, 2009; Seeley 1995, 1997).

Here, we ask whether colony size influences a fundamental attribute of social organization – division of labor – in the harvester ant *Pogonomyrmex californicus*. Broadly defined, division of labor is a statistical pattern in which different individuals perform different functions; it is found, to different degrees, across social groups (reviewed by Michener 1974; Fewell et al. 2009; Holbrook et al. 2009). Division of labor is highly developed in eusocial insect colonies, where it occurs between reproductive and worker castes, and among workers that specialize, over varying time scales, on different non-reproductive tasks (Wilson 1971; Michener 1974; Hölldobler and Wilson 1990, 2009). Functional benefits accrued from division of labor are considered to be of critical importance to the ecological success of social insects (Wilson 1971; Oster and Wilson 1978).

How might division of labor relate to colony size? Ultimate and proximate hypotheses predict that division of labor should increase with colony size. With respect to colony function, the putative benefits of division of labor may be favored more strongly in larger colonies (Bonner 2004), and/or associated costs may select against task specialization in smaller colonies (Oster and Wilson 1978; Herbers 1981; Karsai and Wenzel 1998). Furthermore, self-organizational models of division of labor suggest that increased task specialization may be an emergent consequence of increased colony size (Gautrais et al. 2002; Merkle and Middendorf 2004; Jeanson et al. 2007).

Despite a rich theoretical literature, empirical evidence of colony-size effects on division of labor is scarce. Interspecific surveys of wasps and ants reveal loose, positive correlations between colony size and task specialization (Karsai and Wenzel 1998; Jeanne 2003) or the degree of worker polymorphism, which roughly approximates division of labor (Anderson and McShea 2001; Bonner 2004; but see Fjerdingstad and Crozier 2006). Within social insect species lacking physical worker subcastes, various estimates of task specialization increase with colony size and/or age (Polybia occidentalis: Jeanne 1986; Pogonomyrmex barbatus: Gordon 1989; Rhytidoponera metallica: Thomas and Elgar 2003), although there is only a weak effect on division of labor in the ant *Temnothorax albipennis* (Dornhaus et al. 2009). None of those studies, however, distinguished between effects of colony size and colony age, which are typically correlated but may differ in their relative influence on colony organization (Wilson 1983). Moreover, between-colony comparisons may fail to detect ontogenetic changes in behavior, due to confounding sources of variation.

We examined how the organization of work scales with colony size within and among colonies of *P. californicus*, which are founded independently (without workers) by one or more queens and grow to contain up to several thousand monomorphic workers at maturity (Johnson 2000a). First, we conducted a longitudinal study of growing colonies, to test for early ontogenetic changes while controlling for between-colony variation. Second, we observed unmanipulated, same-aged colonies that varied in size due to differences in growth rate, providing a natural experiment for measuring effects of colony size, independent of colony age. In each case, we quantified non-reproductive division of labor, along with two related components of work that may be size-dependent: (1) the proportional allocation of workers to tasks, which can shift according to colony needs (Gordon 1996; Mailleux et al. 2003; Thomas and Elgar 2003; Tschinkel 2006), and (2) per capita activity, which is predicted to decrease in larger colonies, in conjunction with the scaling of task demand and/or energy use (Jeanson et al. 2007; Hou et al. 2010; Waters et al. 2010). Finally, the rate of encounter between nestmates is hypothesized to scale with colony size and to underlie size-related changes in task organization (Gordon 1996; Pacala et al. 1996); therefore, we analyzed variation in per capita encounter rate as a function of colony size.

Methods

Collections and colony maintenance

Newly-mated *P. californicus* foundresses were collected on 4-6 July 2007 and 4 July 2008, in Pine Valley, San Diego Co., California (32°49’20”N, 116°31’43”W, 1136 m elevation). Because queens of that population are pleometrotic (found colonies cooperatively), laboratory colonies were initiated with two (in 2008) or three (in 2007) randomly chosen queens each. Some queens died during colony founding; the number of queens per established colony ranged from one to three and did not differ significantly between years (t-test: $t_{19} = 1.96$, $P = 0.06$). Colonies were reared at 30°C in plastic nest containers supplied with water (in test tubes plugged with cotton) and *ad libitum* Kentucky bluegrass seeds and previously frozen crickets or mealworms.

Longitudinal study: 3 mo vs. 1 yr

To test for ontogenetic changes in the organization of work during early colony growth, we quantified task performance in seven colonies 3 mo after colony founding and again at 1 yr of age. All colonies were founded in 2008. One week before each observation period, each colony was transferred to an observation nest consisting of two plastic containers (11×11×3.5 cm each) connected by clear vinyl tubing (0.6-cm diameter). One nest container was lined with plaster to retain moisture; colonies kept all brood in this chamber. The other nest container opened, via a 0.6-cm-diameter entrance/exit, into a 55×36-cm foraging arena supplied with water (in a test tube plugged with cotton) and *ad libitum* Kentucky bluegrass seeds. Nests were maintained at 28-30°C under ambient light.

Two days before a colony was observed, all ‘mature’ (darkly pigmented) workers were transferred to a holding container, from which 36 individuals (or all individuals when there were fewer than 36) were essentially randomly selected and marked with a unique color combination on the head, mesosoma, and gaster using Sharpie® oil-based paint markers. Different samples of workers were selected at 3 mo and 1 yr. ‘Callow’ (lightly pigmented) workers were not marked or observed, to avoid potentially confounding effects of worker age structure on division of labor. Each individual was weighed to the nearest 0.1 mg before marking (in other lab colonies, *wet mass* scales with *head width*$^{2.4}$; log-log regression: $R^2 = 0.76$). All workers, marked and unmarked, were returned to the nest approximately 40 hr prior to observation; colonies qualitatively resumed pre-disturbance behavior within several hr.

We conducted 30 scan samples per colony (Altmann 1974), separated by at least 15-min intervals, between 8:00 and 17:00 hrs for one day. To stimulate foraging, we supplemented seeds with previously frozen fruit flies in proportion to the number of workers (~1 fly per 10 workers) at 8:00 hrs. During each sample, we systematically scanned from one corner of the brood chamber to the far end of the foraging arena, noting the behavioral state and location of each marked worker at the instance when she was first seen; after this initial sweep, we searched for specific individuals that had been overlooked. Some individuals could not be found during all 30 scan samples, but each marked worker was observed at least 25 times. Behavioral acts were grouped into five major tasks:

Allogrooming: grooming another worker or queen.

Brood care: antennating, grooming, manipulating, or carrying egg, larva, or pupa; feeding larva.

Food processing: antennating, manipulating, or carrying seed or fly in nest.

Foraging: antennating, manipulating, or carrying seed or fly in foraging arena.

Waste management: antennating, manipulating, or carrying refuse or dead worker.

Other behaviors were scored as undifferentiated activity (could not be assigned to a specific task; e.g., walking, antennating adult nestmate) or inactivity (including self-grooming and receiving allogrooming). Foraging and waste management were likely underestimated because our definitions conservatively excluded workers that were walking in the foraging arena but not contacting food or waste. Following observation, all queens, workers, and brood were removed from the nest, counted, and weighed by caste/developmental stage. Colonies were housed in long-term maintenance nests between observation periods.

Between-colony comparison at 1 yr

To test for effects of colony size, independent of colony age, we utilized natural size variation among 21 unmanipulated, 1-yr-old colonies that differed in net growth rate. Ten of the colonies were founded in 2007; 11 were founded in 2008. Seven of the latter were those also observed at 3 mo. We followed the same protocol as above, except we performed 60 scan samples per colony across two days (30 samples per day; at least 50 total observations per worker). Fruit flies were provided at the beginning of each day.

In addition, to determine whether the rate of encounter between nestmates varied as a function of colony size, we video-recorded colonies for 1 hr between 12:00 and 14:00 hrs on the first day of observation. A Canon GL2 digital video camcorder was focused on the entire brood chamber. During playback, we randomly selected 20 focal workers per colony by imposing a grid on a still image and using a random number generator; five workers each were selected at 0, 15, 30, and 45 min to control for temporal variation in activity. Each individual was observed continuously for 2 min. An encounter was scored when the focal worker came within one antenna-length of another worker. Encounter rate (min^{-1}) was averaged across workers in each of 20 colonies (one recording was accidentally erased). We also used the still images to estimate local density, defined as the number of workers in the brood chamber divided by the number of 1-cm^2 squares occupied (out of 121), averaged across the four images per colony.

Data analysis

Division of labor was quantified using the $\text{DOL}_{\text{indiv-tasks}}$ index, which represents the extent to which individuals specialize on a subset of tasks, or in information-theory terms, the degree to which knowing the identity of an individual predicts the task it performs (Gorelick et al. 2004). When there are more individuals than tasks, $\text{DOL}_{\text{indiv-tasks}}$ can range from 0 (no division of labor) to 1 (maximal division of labor) and is insensitive to changes in the number of individuals, thus permitting statistical comparison across groups of varying size (Gorelick et al. 2004; Jeanson et al. 2007; Jeanson and Fewell 2008; Dornhaus et al. 2009). For each colony, we generated an *individual×task* data matrix, using the proportions of observations during which each individual performed each of the five tasks: allogrooming, brood care, food processing, foraging, or waste management. Next, we normalized the data matrix so that the sum of all entries equaled 1. From the normalized matrix, we calculated Shannon's index, or marginal entropy of tasks (H_{tasks}):

$$H_{\text{tasks}} = -\sum_{j=1}^{m} p_j \log p_j \,,$$

where p_j is the probability that any individual performed the j^{th} task. We then calculated mutual entropy between individuals and tasks ($I_{\text{indiv,tasks}}$), given by

$$I_{\text{indiv,tasks}} = \sum_{i=1,j=1}^{n,m} p_{ij} \log\left(\frac{p_{ij}}{p_i\, p_j}\right),$$

where p_i is the probability that the i^{th} individual performed any task, and p_{ij} is the joint probability that the i^{th} individual performed the j^{th} task. Finally, $\text{DOL}_{\text{indiv-tasks}}$ is defined as

$$\text{DOL}_{\text{indiv-tasks}} = \frac{I_{\text{indiv,tasks}}}{H_{\text{tasks}}}.$$

For the full derivation, see Gorelick et al. (2004), but note that the definitions of $\text{DOL}_{\text{indiv-tasks}}$ and $\text{DOL}_{\text{tasks-indiv}}$ are switched (Dornhaus et al. 2009).

We used paired *t*-tests to analyze within-colony differences (3 mo vs. 1 yr) in $\mathrm{DOL}_{\text{indiv-tasks}}$, proportional task allocation (proportions of colony observations during which workers were engaged in each of the five tasks, and their sum), and per capita activity (total task performance plus undifferentiated activity). Across 1-yr-old colonies, we used simple linear regression to test for effects of colony size (number of workers) on the same response variables, plus per capita encounter rate. Proportions were arcsine-transformed to improve normality. Individuals that died or lost their paint marks were excluded. Within each series of multiple tests, we controlled the false discovery rate using the B-Y method (adjusted $\alpha = 0.018$), which is more powerful than Bonferroni corrections for familywise error rate and does not assume that tests are independent (Benjamini and Yekutieli 2001; Nakagawa 2004; Narum 2006). Analyses were performed using STATISTICA (StatSoft, Inc.). Means ($\overline{X}$) are reported ± 1 standard error.

Results

Longitudinal study: 3 mo vs. 1 yr

Colonies increased in size from 10-30 workers at 3 mo to 160-337 workers at 1 yr of age. Worker age structure did not change over time (paired *t*-test: $t_6 = 0.95$, $P = 0.38$; $\overline{X}_{3\text{mo}} = 7.6 \pm 4.6\%$ callows, $\overline{X}_{1\text{yr}} = 3.0 \pm 0.7\%$ callows), but the brood-to-worker ratio was higher in 1-yr-old colonies ($t_6 = 4.52$, $P = 0.004$; $\overline{X}_{3\text{mo}} = 0.6 \pm 0.1$ mg/worker, $\overline{X}_{1\text{yr}} = 1.1 \pm 0.1$ mg/worker). Average worker body size was larger at 1 yr ($t_6 = 5.58$, $P = 0.001$; grand means: $\overline{X}_{3\text{mo}} = 2.1 \pm 0.1$ mg, $\overline{X}_{1\text{yr}} = 2.8 \pm$

0.1 mg); however, colony age did not affect the coefficient of variation of body size (t_6 = 0.02, P = 0.99).

Division of labor ($\text{DOL}_{\text{indiv-tasks}}$) was higher at 1 yr than at 3 mo in six of seven colonies, a significant effect (paired t-test: t_6 = 4.08, P = 0.006; $\overline{X}_{3\text{mo}}$ = 0.25 ± 0.02, $\overline{X}_{1\text{yr}}$ = 0.37 ± 0.01; Fig. 4.1). Colony-level task allocation also shifted; older, larger colonies performed relatively less brood care, but colony age did not significantly affect proportional allocation to the other tasks, total task performance, or per capita activity (Table 4.1). Intracolonial distributions of individual activity were left-skewed, with over 90% of workers active during at least half of observations; neither skewness (t_6 = 0.69, P = 0.51) nor the coefficient of variation (t_6 = 1.19, P = 0.28) differed between 3 mo and 1 yr.

Between-colony comparison at 1 yr

Colony size ranged from 30 to 390 workers and was not affected by the year of colony founding (t-test: t_{19} = 0.98, P = 0.34) or the number of queens (ANOVA: $F_{2,18}$ = 0.98, P = 0.39); colonies were pooled for further analysis. Variation in colony size was not associated with the proportion of callow workers (linear regression: $F_{1,19}$ = 0.04, P = 0.85, R^2 = 0.002) or the brood-to-worker ratio ($F_{1,19}$ = 0.10, P = 0.75, R^2 = 0.005). There was a non-significant trend of increasing worker body size with colony size ($F_{1,19}$ = 4.06, P = 0.06, R^2 = 0.18), but no effect of colony size on the coefficient of variation of body size ($F_{1,19}$ = 0.14, P = 0.71, R^2 = 0.007).

Division of labor ($DOL_{indiv\text{-}tasks}$) increased with colony size across 1-yr-old colonies (linear regression: $DOL_{indiv\text{-}tasks} = 0.23 + 2.9\times10^{-4} worker\ number$; $F_{1,19} = 11.25$, $P = 0.003$, $R^2 = 0.37$; Fig. 4.2). There was no effect of queen number on $DOL_{indiv\text{-}tasks}$ (ANOVA: $F_{2,18} = 0.53$, $P = 0.60$). Colony size did not affect the proportional allocation of workers to tasks, total task performance, or per capita activity (Table 4.2). Intracolonial activity distributions were not related to colony size (skewness: $F_{1,19} = 0.99$, $P = 0.33$, $R^2 = 0.05$; coefficient of variation: $F_{1,19} = 0.03$, $P = 0.86$, $R^2 = 0.002$).

The per capita rate of encounter between nestmates increased with colony size (linear regression: $encounters/min = 4.27 + 0.014 worker\ number$; $F_{1,18} = 16.02$, $P = 0.001$, $R^2 = 0.47$; Fig. 4.3). Although nest size was held constant, local density within the brood chamber increased more slowly than colony size ($\log_{10} workers/cm^2 = 0.03 + 0.14 \log_{10} worker\ number$; slope vs. 1: $t_{18} = 22.81$, $P < 0.0001$, $R^2 = 0.45$); in other words, a 10-fold increase in colony size resulted in just a 1.4-fold increase in local density. This allometry was caused by aggregation of workers in small colonies, rather than an inverse relationship between colony size and the proportion of workers residing inside the nest ($F_{1,18} = 3.27$, $P = 0.09$, $R^2 = 0.15$). As a consequence, variation in encounter rate was not explained by variation in local density ($F_{1,18} = 0.63$, $P = 0.43$, $R^2 = 0.03$).

Discussion

The extension of biological scaling beyond organisms implies that the size of a social group can affect the structure, function, and ecology of its individual members and of the group as a whole (Bonner 2004; Yip et al. 2008). Social scaling relations potentially occur in groups of all forms, but size seems to be especially important for the organization of highly integrated, eusocial colonies (Tschinkel 1993; Bourke 1999; Anderson and McShea 2001; Jeanne 2003; Hou et al. 2010; Waters et al. 2010). Here, we show that division of labor, a fundamental colony-level phenotype, scales positively with colony size in the harvester ant *P. californicus*; individual workers in larger (and older) colonies specialize on narrower subsets of tasks. This relationship is found in two different contexts: (1) during early colony ontogeny or sociogenesis, as a colony grows from tens to hundreds of workers, and (2) across colonies that vary in size, independently of age.

Does this consistent scaling pattern have adaptive significance? Division of labor is generally thought to enhance colony 'efficiency' and thus fitness; benefits may include individual learning gains, reduction of task switching costs, and the evolution of specialized morphological or physiological 'machinery' (Smith 1776; Oster and Wilson 1978; Dornhaus 2008; Chittka and Muller 2009). Actual advantages have rarely been verified, with most tests focusing on the rate or speed of individual performance, not the ultimate conversion of labor and resources into sexual offspring (Wilson 1980; Porter and Tschinkel 1985;

Tschinkel 1993; Dukas and Visscher 1994; Chittka and Thomson 1997; Trumbo and Robinson 1997; Julian and Cahan 1999; Dornhaus 2008). Assuming colony-level benefits exist, they may be more important to larger colonies, if increased size imposes physical or ecological constraints (Jun et al. 2003; Bonner 2004). Division of labor may also confer costs that outweigh its benefits in smaller colonies; for instance, the need for specific tasks to be performed may be too low and/or variable to keep task specialists employed (Bell and Mooers 1997; Karsai and Wenzel 1998), or individual specialization may reduce system reliability when worker redundancy is low (Oster and Wilson 1978; Herbers 1981). Alternatively, the scaling of division of labor could be an emergent epiphenomenon with little, if any, adaptive value (Gautrais et al. 2002; Merkle and Middendorf 2004; Jeanson et al. 2007).

Our study did not directly address those hypotheses, but it does provide relevant insights. First, if the observed scaling relationship is driven by functional limitations or costs in small colonies, then division of labor might be expected to stabilize once a critical colony size is attained; yet, it appears to increase continuously throughout the range of colony sizes we measured (Fig. 4.2). This raises the question of how colonies respond to further increases in size, which can reach several thousand workers in full-grown colonies (Johnson 2000a). Second, Waters et al. (2010) demonstrated that whole-colony metabolic rate scales with negative allometry in *P. californicus* (i.e., larger colonies use less energy per gram of worker). Perhaps higher division of labor enables larger colonies to operate more efficiently, reducing their relative energy requirements. Or, metabolic scaling may arise from energetic constraints imposed by increased colony size, which could simultaneously favor or even necessitate increased division of labor (Bonner 2004).

The scaling of division of labor could possibly be mediated by underlying changes in worker body size. Body size is correlated with task performance in ants with polymorphic workers (Oster and Wilson 1978; Hölldobler and Wilson 1990), and to a lesser extent, in some social insects lacking distinct physical worker subcastes (Schwander et al. 2005; Jandt and Dornhaus 2009). Moreover, in polymorphic ants, the range of worker sizes can expand during colony ontogeny, potentially facilitating division of labor (Wilson 1983; Tschinkel 1988, 1993). Although workers of *P. californicus* are monomorphic (i.e., body size is normally distributed), average body size increased between 3 mo and 1 yr of colony age, and there was a weak trend of increasing body size with colony size among same-aged colonies. Worker size variability, however, was not related to colony age or size.

Colony-size effects on task allocation and activity

Colony needs or priorities may covary with colony size, causing size-related changes in the allocation of workers across tasks (Gordon 1996; Mailleux et al. 2003; Thomas and Elgar 2003; Tschinkel 2006). For example, smaller colonies may invest relatively more effort in tasks promoting colony growth, such as brood care and foraging (Kolmes and Winston 1988; Schmid-Hempel et al. 1993; Thomas and Elgar 2003; Tschinkel 2006). In our longitudinal comparison, younger and smaller *P. californicus* colonies performed proportionally more brood care, even though they contained less brood mass per worker. In contrast, task allocation did not shift with colony size among 1-yr-old colonies. This discrepancy indicates that ontogenetic changes in the organization of work can differ from effects of colony size alone, and highlights the importance of distinguishing within- vs. between-colony scaling relations.

Social insect workers spend much of their time either inactive or engaged in undifferentiated activity not associated with a specific task (Lindauer 1961; Cole 1986; Schmid-Hempel 1990). Does activity or workload vary predictably with colony size? If economies of scale reduce relative task demand (Jeanson et al. 2007) and/or energy expenditure in larger colonies (Hou et al. 2010; Waters et al. 2010), then the average individual workload is expected to decrease with increasing colony size. Yet, neither of our measures of per capita activity – based strictly on task performance or including all activity – changed with colony size. A previous study that quantified locomotory activity in *P. californicu*s likewise found no colony-size effect on average worker speed, although larger colonies exhibited a greater disparity between fast and slow individuals (Waters et al. 2010). Colony size has an opposite effect in *T. albipenis*; intracolonial distributions of workload during nest emigration are more skewed in smaller colonies (Dornhaus et al. 2008). We did not detect any relationship between colony size and the distribution of activity across workers.

Size-dependency of encounter rate

In functionally integrated, eusocial insect colonies, workers communicate task needs and opportunities through dense networks of social interaction (Wilson and Hölldobler 1988; Seeley 1995; Gordon 1996; Fewell 2003; Hölldobler and Wilson 2009). Size-dependent changes in the rate of encounter between nestmates could thus modulate information flow and the organization of work (Gordon 1996; Pacala et al. 1996). Encounter rate may also provide a cue of colony size or density that influences workers' task decisions (Pratt 2005). In our study, per capita encounter rate scaled positively with colony size. Although nest size was held constant, the aggregation of workers in small colonies caused worker density to increase more slowly than colony size; as a result, variation in encounter rate was not explained by variation in density alone. This pattern is consistent with previous findings in other ants. Workers of *Lasius fuliginosus* cluster together when global density is low, elevating their local rate of encounter (Gordon et al. 1993). Encounter rate also increases with colony size (and task specialization), independently of density, in *Rhytidoponera metallica* (Thomas and Elgar 2003). It remains unclear, however, whether encounter rate and division of labor are functionally linked, or respond separately to changes in colony size.

Most laboratory studies, including our own, employ simplified nest designs that may reduce spatial segregation between workers and/or between tasks. Spatial task-encounter models (Tofts and Franks 1992) and empirical evidence of worker spatial fidelity within nests (Sendova-Franks and Franks 1994; Tschinkel 2004) suggest that nest architecture can contribute to patterns of social interaction and task organization. Spatial constraints are implicated in many biological scaling phenomena (Brown and West 2000; Yip et al. 2008); potential interactions between colony size, spatial structure, and division of labor merit further research.

Conclusions

Changes in size have broad implications for the form and function of biological entities, across levels of organization (Peters 1983; Schmidt-Nielsen 1984; Tschinkel 1993; Brown and West 2000; Bonner 2004; Yip et al. 2008). The observed relationship between size and division of labor in social insect colonies parallels the scaling of physiological division of labor with body size in multicellular organisms, which, like colonies, are collectives of lower-level subunits (Bell and Mooers 1997; Bonner 2004). Task specialization increases with population size in many human organizations as well (Smith 1776; Young 1928; Changizi et al. 2002; Bonner 2004) . These patterns are undoubtedly generated by different mechanisms, but their convergence may reflect common selective pressures (natural or economic) and/or self-organizing processes.

Although division of labor has been evolutionarily elaborated in eusocial insects, it remains a basic attribute of sociality that is expressed by groups of varying size, composition, and complexity (Michener 1974; Fetherston et al. 1990; Stander 1992; Lacey and Sherman 1997; Underwood and Shapiro 1999; Clutton-Brock et al. 2003; Arnold et al. 2005; Gazda et al. 2005; Ridley and Raihani 2008; Fewell et al. 2009; Holbrook et al. 2009; Bruintjes and Taborsky 2010). Size increase can promote task specialization in smaller, less integrated social groups, such as cooperative colony-founding associations of ant queens (Jeanson and Fewell 2008). We advocate further investigations of social scaling, of division of labor and other traits, within and among diverse systems. The aim of this pursuit is twofold: to gain novel insights into the social organization, evolution, and ecology of specific taxa, and to determine whether sociobiology is governed by, or gives rise to, general scaling principles.

Table 4.1. Proportional task allocation and per capita activity in *P. californicus* colonies at 3 mo (10-30 workers) vs. 1 yr of age (160-337 workers).

	Proportion of observations			
Task	3 mo	1 yr	t_6	*P*
Allogrooming	0.044 ± 0.003	0.036 ± 0.006	1.06	0.33
Brood care	0.118 ± 0.014	0.062 ± 0.011	3.80	0.009*
Food processing	0.218 ± 0.024	0.235 ± 0.022	0.54	0.61
Foraging	0.007 ± 0.001	0.019 ± 0.007	1.93	0.10
Waste management	0.025 ± 0.008	0.062 ± 0.008	2.55	0.04
Total task performance	0.411 ± 0.025	0.414 ± 0.023	0.11	0.92
Total activity	0.746 ± 0.023	0.828 ± 0.019	2.27	0.06

Proportion of observations is mean ± SE proportion of colony observations during which workers performed tasks or were active. Total task performance is sum of five tasks. Total activity is total task performance plus undifferentiated activity. *t* is from paired *t*-test. B-Y adjusted $\alpha = 0.018$ (* denotes significance).

Table 4.2. Proportional task allocation and per capita activity regressed against colony size in 1-yr-old *P. californicus* colonies (30-390 workers).

	Regression vs. colony size		
Task	R^2	$F_{1,19}$	P
Allogrooming	<0.001	0.004	0.95
Brood care	0.08	1.63	0.22
Food processing	0.006	0.11	0.75
Foraging	0.02	0.32	0.58
Waste management	0.09	1.99	0.17
Total task performance	0.01	0.21	0.65
Total activity	0.01	0.21	0.65

For each response variable, results are from simple linear regression of arcsine-transformed proportion of colony observations on number of workers. Total task performance is sum of five tasks. Total activity is total task performance plus undifferentiated activity. B-Y adjusted $\alpha = 0.018$.

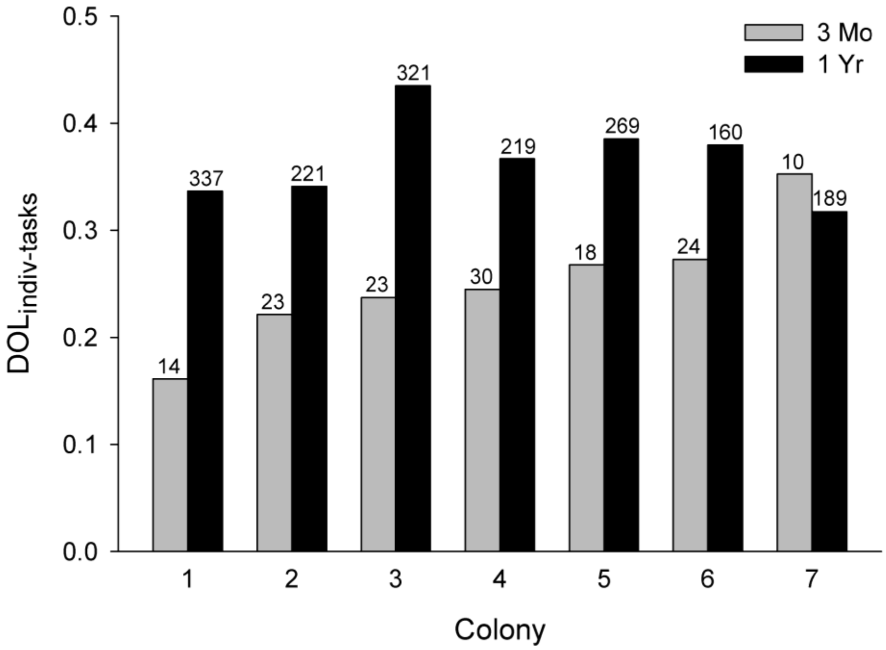

Figure 4.1. Division of labor in *P. californicus* colonies at 3 mo vs. 1 yr of age. $\mathrm{DOL}_{\text{indiv-tasks}}$ represents the degree to which individuals specialize on a subset of tasks. Values above bars are numbers of workers in each colony at time of observation.

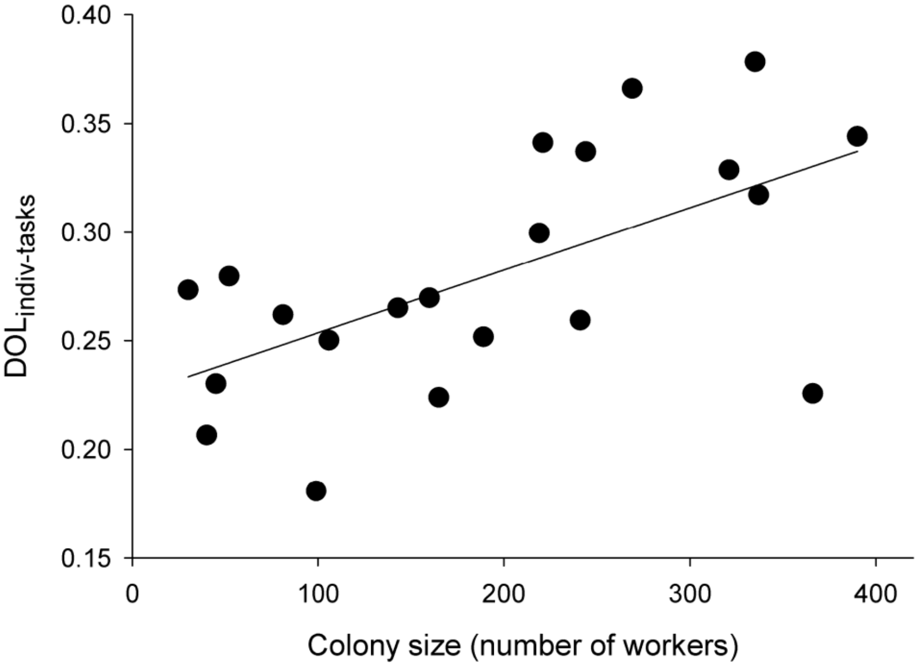

Figure 4.2. Division of labor as a function of colony size in 1-yr-old *P. californicus* colonies. $DOL_{indiv\text{-}tasks}$ represents the degree to which individuals specialize on a subset of tasks. Ordinary least squares regression line is shown ($Y = 0.23 + 2.9\times10^{-4}X$; $R^2 = 0.37$).

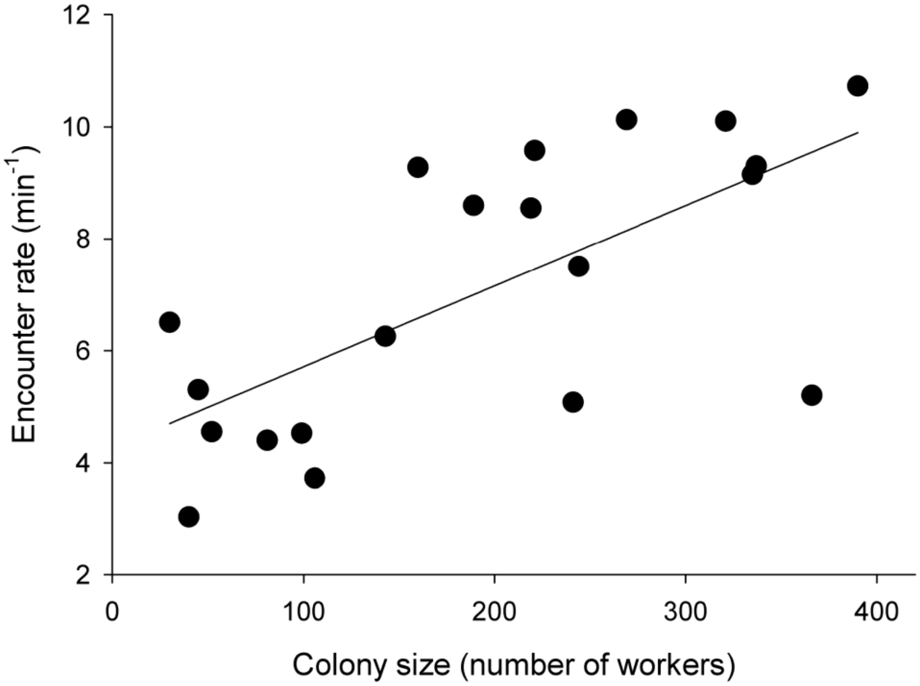

Figure 4.3. Colony size vs. per capita rate of encounter between nestmates in 1-yr-old *P. californicus* colonies. Encounter rate (min^{-1}) is average of 20 focal workers per colony. Ordinary least squares regression line is shown ($Y = 4.27 + 0.014X$; $R^2 = 0.47$).

CHAPTER 5

HARVESTER ANTS SHIFT TASK ALLOCATION, BUT NOT SPECIALIZATION, IN RESPONSE TO SHORT-TERM CHANGES IN COLONY SIZE

Abstract: Colony size plays an important role in the organization of insect societies. In the harvester ant *Pogonomyrmex californicus,* division of labor increases with colony size, both during colony ontogeny and among unmanipulated colonies of the same age. However, the mechanism(s) integrating individual task specialization and colony size is unknown. Self-organizational models predict that higher division of labor should emerge spontaneously as colony size increases. Alternatively, colony-size effects on division of labor may be mediated by developmental processes that require longer-term size differentiation. We manipulated colony size in *P. californicus* and quantified task performance over several days. Experimental variation in colony size failed to elicit a short-term response in division of labor; therefore, we propose that the previously observed scaling relationship is a product of functional integration, rather than a purely emergent epiphenomenon. In contrast, changes in colony size caused rapid shifts in the proportional allocation of workers to tasks, suggesting that colony needs or task priorities depend, in part, on colony size alone. Finally, although task allocation was flexible, colony members differed consistently in

task performance and spatial tendency across colony size treatments. One source of behavioral variability was worker age; another may be genotypic diversity.

Introduction

The outstanding ecological success of eusocial insects, such as ants, bees, wasps, and termites, can be partly attributed to their efficient organization of work (Oster and Wilson 1978). A primary, colony-level pattern of organization is division of labor, expressed not only between reproductive and worker castes, but also among workers that specialize on different tasks (Wilson 1971; Michener 1974; Hölldobler and Wilson 1990, 2009). Non-reproductive division of labor, or polyethism, is thought to enhance colony performance and ultimately fitness (Oster and Wilson 1978; Chittka and Muller 2009; but see Dornhaus 2008). However, colonies must balance the putative benefits of division of labor with the need to allocate workers in accordance with task demand, which varies depending on internal and external conditions (Calabi and Traniello 1989; Seeley 1995; Gordon 1996). The purpose of this study is to investigate how task specialization and allocation are integrated with a fundamental colony attribute: colony size.

Within eusocial insect colonies, interindividual variation in task performance is correlated or causally linked with differences in worker age, experience, morphology, physiology, genotype, and gene expression (reviewed by Oster and Wilson 1978; Hölldobler and Wilson 1990, 2009; Robinson 1992; Beshers and Fewell 2001; Smith et al. 2008; Robinson 2009). A leading phenomenological model of division of labor – the response threshold model – proposes that these intrinsic factors affect individual responsiveness to extrinsic, task-related stimuli (Robinson and Page 1989; Bonabeau et al. 1996; Page and Mitchell 1998; Theraulaz et al. 1998; Beshers and Fewell 2001). If workers vary in their response thresholds for different tasks, and if the performance of a task reduces the intensity of an associated stimulus, then a division of labor can emerge in which workers with the lowest thresholds for a given task become specialists for that task. The response threshold model also offers a basic explanation for how workers can be allocated to meet colony needs. When a change in the social or physical environment increases demand for a task, its stimulus level should exceed the thresholds of additional workers, recruiting a broader subset of the colony to perform the task. This prediction has been supported by experimental manipulations of task demand (Detrain and Pasteels 1991, 1992; Fewell and Page 1993; O'Donnell and Foster 2001; Weidenmuller 2004).

Worker behavior can also be modulated by colony state parameters, including colony size, demography, nutritional status, and developmental phase (Oster and Wilson 1978; Wilson 1985; Robinson 1992; Schmid-Hempel et al. 1993; Anderson and McShea 2001). Colony size, which typically increases during colony ontogeny and can be abruptly diminished by natural disturbance or reproduction events (Wilson 1971), appears to exert a strong influence on the organization of work; positive relationships between colony size/age and the degree of task specialization have been observed in several species of ants and wasps (Jeanne 1986; Gordon 1989; Thomas and Elgar 2003; Holbrook et al. in press; but see Dornhaus et al. 2009). In the harvester ant *Pogonomyrmex californicus*, workers are more specialized in older, larger colonies, and division of labor increases with colony size independently of colony age (Holbrook et al. in press). However, the mechanism(s) of integration between task specialization and colony size is unknown.

Simulations based on the fixed response threshold model, in which individual thresholds are invariable over time, suggest that the scaling of division of labor can be an emergent epiphenomenon (Jeanson et al. 2007). Increased group size spontaneously promotes higher division of labor in artificially-established groups of normally solitary ant foundresses (Jeanson and Fewell 2008) and sweat bees (Chapter 3), but this ‘null’ hypothesis has not been tested in larger and more complex eusocial colonies. An alternative, though not mutually exclusive, explanation is that colony-size effects on division of labor are mediated by developmental processes that require longer-term size differentiation. For example, self-reinforcement of response thresholds may cause workers in larger colonies to become more specialized over time (Theraulaz et al. 1998; Gautrais et al. 2002; Merkle and Middendorf 2004), or division of labor may be functionally regulated by colony-level control mechanisms (Wilson 1985; Beshers et al. 1999). Moreover, colony needs may covary with colony size, triggering shifts in the proportional allocation of workers to tasks and possibly altering the opportunity for task specialization (Kolmes and Winston 1988; Schmid-Hempel et al. 1993; Thomas and Elgar 2003; Tschinkel 2006; Holbrook et al. in press).

The relationship between colony size and division of labor has been investigated only in unmanipulated colonies, making it difficult to distinguish among potential proximate causes (Jeanne 1986; Gordon 1989; Thomas and Elgar 2003; Holbrook et al. in press). Here, we experimentally manipulated colony size in *P. californicus,* controlling for other sources of between-colony variation. If the scaling of division of labor is primarily an emergent epiphenomenon (Jeanson et al. 2007), then a sudden change in colony size should elicit a short-term response. If developmental processes are involved, then a longer time lag is expected for the reorganization of work. We observed the same workers in both small and large colonies so that colony-level responses could be explained by underlying, individual-level changes in task performance.

Methods

Source colonies

Newly-mated *P. californicus* foundresses were collected on 4 July 2008 in Pine Valley, San Diego Co., California (32°49’20”N, 116°31’43”W, 1136 m elevation). Because queens of that population are pleometrotic (found colonies cooperatively), laboratory colonies were initiated with two queens each. Colonies were reared at 30°C in plastic nest containers supplied with water (in test tubes plugged with cotton) and *ad libitum* Kentucky bluegrass seeds and pieces of previously frozen crickets or mealworms.

Experimental procedure

To test for short-term effects of colony size on the organization of work, we manipulated colony size and quantified task performance over a time span of several days. Ten source colonies were subjected to sequential size manipulations of 50 workers (‘small colony’) vs. 300 workers (‘large colony’). In five, randomly-assigned source colonies, we applied the small colony size treatment first, followed by the large colony size treatment; the other five source colonies were tested in reverse order (from large to small). This paired design controlled for potentially confounding sources of between-colony variation (e.g., genetic, demographic, etc.). Source colonies were 21-22 mo old and contained two queens and between 355-835 workers.

Two days before each colony was tested, we individually marked 36 workers per colony with a unique color combination on the head, mesosoma, and gaster using Sharpie® oil-based paint markers. Darker, ‘mature’ workers and more lightly pigmented, ‘callow’ workers were sampled in proportion to their composition of the source colony (average ± SD = 10 ± 4% callow); within each of the two broadly-defined age cohorts, focal individuals were essentially randomly selected from a holding container. Callow workers were approximately 3-10 days old; mature workers had lived from two weeks up to several months.

One hr after marking workers, we established an experimental colony with one queen, either 50 or 300 workers including all marked workers, and brood. The proportion of callow workers and the ratios of pupae-mass-to-worker-number and larvae-mass-to-worker-number in each experimental colony were set to match the composition of the respective source colony (average ± SD = 0.5 ± 0.2 mg pupae/worker; 0.6 ± 0.2 mg larvae/worker). Unmarked workers and brood were essentially randomly selected. To control for nestmate density, small and large experimental colonies were housed in different-sized, plastic observation nests (small = 11 x 11 x 3.5 cm; large = 31 x 23 x 10 cm; surface density = 0.4 workers/cm^2; ants could not climb walls). Each nest was lined with plaster and opened, via a 0.6-cm-diameter entrance/exit, into a 55 x 36-cm foraging arena provisioned with water (in a test tube plugged with cotton) and *ad libitum* Kentucky bluegrass seeds. Observation nests were maintained at 28-30°C under ambient light.

Behavioral observations were initiated after an approximately 36-hr acclimation period; colonies qualitatively resumed pre-disturbance behavior within several hr. We conducted 50 scan samples per colony, separated by at least 20-min intervals, between 7:00 and 19:00 hrs for two days (25 samples per day). During each sample, we systematically scanned from one corner of the nest to the far end of the foraging arena, recording the behavioral state and location of each marked worker at the instance when she was first seen; after this initial sweep, we searched for specific individuals that had been overlooked. A minority of individuals was not found during all scan samples, but each worker was observed at least 41 times. Behavioral acts were grouped into five major tasks:

Allogrooming: grooming another worker or queen.

Brood care: antennating, grooming, manipulating, or carrying egg, larva, or pupa; feeding larva.

Food processing: antennating, manipulating, or carrying seed or fly in nest.

Foraging: antennating, manipulating, or carrying seed or fly in foraging arena.

Waste management: antennating, manipulating, or carrying refuse or dead worker.

Foraging and waste management were likely underestimated because our definitions of those tasks conservatively excluded workers that were walking in the foraging arena but not contacting food or waste. Thus, we also noted whether each focal individual was located inside or outside of the nest, the latter indicating higher potential to perform outside-nest tasks. At the beginning of each day of observation, seeds were supplemented with ~1 frozen fruit fly per 10 workers to stimulate foraging.

Immediately following behavioral observations, each experimental colony was recombined with its source colony, and a smaller (50 workers) or larger (300 workers) experimental colony was formed following the procedure described above. New experimental colonies were allowed to acclimate for approximately

36 hr. We then conducted another set of 50 scan samples across two days, as before. The same focal individuals were observed under each colony size treatment, allowing us to analyze individual behavioral changes underlying colony responses.

Data analysis

Division of labor was quantified using the $\mathrm{DOL}_{\text{indiv-tasks}}$ index, representing the extent to which individuals specialize on a subset of tasks, or in information theory terms, the degree to which knowing the identity of an individual predicts the task it performs (Gorelick et al. 2004; Jeanson et al. 2007; Dornhaus et al. 2009). For each colony, we generated a normalized matrix of task performance using the proportions of observations during which each individual was engaged in each of the five tasks: allogrooming, brood care, food processing, foraging, or waste management. $\mathrm{DOL}_{\text{indiv-tasks}}$ was calculated as mutual entropy between individuals and tasks ($I_{\text{indiv,tasks}}$) divided by Shannon's index or marginal entropy of tasks (H_{tasks}). For details, see Gorelick et al. (2004), but note that the definitions of the indices $\mathrm{DOL}_{\text{indiv-tasks}}$ and $\mathrm{DOL}_{\text{tasks-indiv}}$ are switched (Dornhaus et al. 2009). Because our direct quantification of foraging and waste management may have been overly conservative (see above), and those tasks occurred predominantly in the foraging arena, we also calculated a $\mathrm{DOL}_{\text{indiv-loc}}$ index using locations (inside vs. outside of nest) instead of tasks.

We tested for an effect of colony size on each $\mathrm{DOL}_{\mathrm{indiv}}$ index, calculated across tasks and across locations, using repeated measures ANOVA, with colonies as subjects and colony size as a within-subject fixed factor. We used similar rmANOVA models to test for colony-size effects on colony-level task allocation (the proportion of worker-observations allocated to each task, out of all observations and relative to total task performance) and on the proportion of observations during which workers were located outside of the nest. Moreover, when colony size had a significant effect on the collective performance of a specific task, we further tested whether the response resulted from differences in the numbers of workers performing the task (colony-level rmANOVA as above) and/or in the frequency of performance by workers who engaged in the task across colony size treatments (rmANOVA with individuals as subjects, colony as a between-subject blocking factor, and colony size as a within-subject fixed factor; response variables were arcsine-transformed proportions of observations).

To test for between-individual consistency in task performance and location across colony size treatments, we calculated Spearman rank correlations by colony. Ranks were based on the proportion of observations during which each individual was engaged in each task or located outside of the nest. Finally, we performed χ^2 contingency table analyses to determine whether worker age was associated with performance (yes/no) of each of the five tasks and/or tendency to exit the nest. Because the sample size of callow workers per colony was small, individuals were pooled across colonies ($n_{\mathrm{callow}} = 34$, $n_{\mathrm{mature}} = 281$).

Within each series of multiple, related tests, we controlled for the false discovery rate following the B-Y method, which is more powerful than Bonferroni corrections for familywise error rate and does not assume that tests are independent (Benjamini and Yekutieli 2001; Nakagawa 2004; Narum 2006). Individuals that died or lost their paint marks in either colony size treatment were excluded from all tests. Data analysis was performed using STATISTICA (StatSoft, Inc.).

Results

Effects of colony size

There were no consistent effects of colony size on $\mathrm{DOL}_{\text{indiv-tasks}}$, the degree to which individuals specialized on tasks (rmANOVA: $F_{1,9} = 0.73$, $P = 0.41$; Fig. 5.1a), or on $\mathrm{DOL}_{\text{indiv-loc}}$, the degree to which individuals exhibited spatial fidelity inside vs. outside of the nest ($F_{1,9} = 0.19$, $P = 0.67$). $\mathrm{DOL}_{\text{indiv-tasks}}$ varied from 0.24 to 0.36, which is within the range of values measured in unmanipulated *P. californicus* colonies (Holbrook et al. in press).

Colony-level task allocation, however, shifted in response to experimental colony size variation (Table 5.1; Fig. 5.1b-h). Out of all observations, large colonies performed proportionally less brood care but more food processing and waste management, such that total task performance per worker increased with colony size. Colony size did not affect per capita performance of allogrooming or foraging, or the proportion of workers located outside of the nest. Similar effects were found when each task was analyzed as a proportion of the colony's total task performance, thus controlling for variation in overall activity; large colonies allocated relatively more labor to food processing ($F_{1,9}$ = 9.43, P = 0.01) and waste management ($F_{1,9}$ = 10.73, P = 0.01), and relatively less to brood care ($F_{1,9}$ = 32.30, P < 0.001), with no significant differences in allogrooming ($F_{1,9}$ = 2.40, P = 0.16) or foraging ($F_{1,9}$ = 4.05, P = 0.08; B-Y adjusted α = 0.02).

Underlying the collective shifts in brood care and waste management were corresponding changes in the number of engaged workers (brood care: $F_{1,9}$ = 6.10, P = 0.04; waste management: $F_{1,9}$ = 11.61, P = 0.008) and in individual effort, as measured by the frequency of performance by engaged workers (brood care: $F_{1,149}$ = 17.29, P < 0.0001; waste management: $F_{1,71}$ = 15.29, P < 0.001). Food processing was performed by all but one focal individual in small and large colonies, but individuals performed the task more frequently in large colonies ($F_{1,303}$ = 44.93, P < 0.0001).

Between-individual behavioral consistency

While individuals and colonies exhibited plasticity in response to colony size, there was also significant between-individual consistency within source colonies; i.e., individuals that performed a given task more frequently in small colonies also performed it more frequently in large colonies, maintaining their rank order (Table 5.2). Within most colonies, workers exhibited consistent differences in brood care, waste management, total task performance, and outside-nest activity. The rank order of food processors was maintained in just two out of ten colonies, whereas there were no significant intracolonial correlations for allogrooming.

Effects of worker age

One intrinsic factor associated with individual task performance was worker age. Across colony size treatments, callow workers were more likely than mature workers to perform brood care ($\chi^2_1 = 9.91$, $P = 0.002$) and less likely to perform foraging ($\chi^2_1 = 21.92$, $P < 0.0001$) or waste management ($\chi^2_1 = 37.87$, $P < 0.0001$; B-Y adjusted $\alpha = 0.02$; Fig. 5.2). In agreement with these differences in task performance, callow workers were less likely to exit the nest into the foraging arena, where foraging and the majority of waste management occurred ($\chi^2_1 = 123.69$, $P < 0.0001$). Worker age was not associated with the likelihood of allogrooming ($\chi^2_1 = 2.14$, $P = 0.14$) or food processing (performed by all workers). Overall, mature workers performed a greater number of tasks than callow workers (Mann-Whitney *U* test: $U = 2151.5$, $P < 0.0001$). The results did not change when small and large colonies were analyzed separately. Callow workers constituted just 5-16% of colonies; when $\mathrm{DOL}_{\text{indiv-tasks}}$ was calculated using only mature workers, it was still not affected by colony size (rmANOVA: $F_{1,9} = 0.64$, $P = 0.45$). Excluding callow workers changed the outcome of just four between-individual behavioral correlations: interindividual consistency in food processing became significant in one colony and non-significant in another, while the rank order of total task performance was no longer maintained in two colonies.

Discussion

Colony size and division of labor

In an earlier study, we demonstrated that division of labor increases with colony size in the harvester ant *P. californicus*, during early colony ontogeny and independently of colony age (Holbrook et al. in press). Here, experimental manipulation of colony size failed to elicit a short-term change in division of labor. The same range of colony sizes (50-300 workers) is associated with a marked increase in division of labor among unmanipulated *P. californicus* colonies that have undergone long-term size differentiation (Holbrook et al. in press). This suggests that the scaling of division of labor is not an emergent epiphenomenon produced by transitory behavioral responses (Jeanson et al. 2007); instead, colony size appears to have a lasting effect on the degree to which workers specialize.

How might task specialization be integrated with colony size? Workers' response thresholds may be self-reinforced, or modified by task-related experience or learning; for instance, the act of performing a task may reduce an individual's corresponding threshold, increasing the probability of future performance (Theraulaz et al. 1998; Weidenmuller 2004; Ravary et al. 2007). Extended response threshold models suggest that self-reinforcement can indirectly promote higher task specialization in larger colonies, although the dynamics are complex and depend on simulation conditions (Gautrais et al. 2002; Merkle and Middendorf 2004). Alternatively, division of labor may be functionally regulated by control mechanisms operating at the level of the colony. Perhaps the ‘colony threshold distribution’, across workers and tasks, is developmentally modulated by colony size, in a process analogous to the social regulation of physical and temporal worker subcastes in other eusocial systems (Wilson 1983; Wheeler and Nijhout 1984; Wilson 1985; Beshers et al. 1999; Huang and Robinson 1999). Whatever the mechanism – self-reinforcement, social regulation, etc. – it is likely mediated by physiological changes that delay individual responses to colony size, or might even require worker turnover to reorganize division of labor (Robinson 1992; Huang and Robinson 1999; Robinson 2009).

Abrupt worker loss, as simulated in our experiment, can occur naturally in social insects, due to predation, severe weather, nest damage, disease, or colony budding or fission (Wilson 1971). Previous manipulations of colony size have sometimes, but not always, altered colony organization. When Wilson (1983) reduced *Atta cephalotes* leafcutter ant colonies from about 10,000 to 236 workers, the body size distribution of newly-produced workers reverted to that of an incipient colony; effects on task performance were not measured. In another polymorphic ant, *Pheidole morrisi*, short-term changes in colony size alone had no effect on the behavioral repertoire of major workers (Brown and Traniello 1998). And while honey bee (*Apis mellifera)* workers began foraging at younger ages following drastic reductions in colony size (Winston and Fergusson 1985), their temporal caste schedules did not shift in response to more moderate size decreases (Kolmes and Winston 1988). None of those studies quantified division of labor *per se*, which is a colony-level property whereby different individuals perform different tasks (Michener 1974). Further work is needed to understand how, and how quickly, colony size affects the degree of task specialization in eusocial insects. The scaling of division of labor within and among undisturbed colonies may be entangled with a complex array of cofactors, including colony demography, task demand, and social interactions.

Size effects on task allocation

Another important component of the organization of work in insect colonies is the allocation of workers to tasks such that colony needs are satisfied (Calabi and Traniello 1989; Seeley 1995; Gordon 1996). We observed several colony-size-related shifts in overall task allocation. On a per capita basis, large colonies performed less brood care, more food processing, and more waste management than small colonies. In contrast, proportional allocation to allogrooming, foraging, and total outside-nest activity were not affected by colony size. Changes in collective task performance can occur in two ways: through variation in the numbers of workers engaged in specific tasks and/or through changes in individual task effort (Fewell and Winston 1992; Schmid-Hempel et al. 1993). Both mechanisms were utilized by *P. californicus*. In large colonies, fewer individuals performed brood care, and those who did performed it less frequently (i.e., lower individual effort) than in small colonies. The inverse was true for waste management: more workers performed the task more frequently in large colonies. Food processing was performed by nearly all individuals in both small and large colonies, but individual effort was higher in large colonies. Overall, workers were more active (i.e., performed tasks more often) in larger colonies. The distribution of task performance in manipulated *P. californicus* colonies was comparable to that exhibited by unmanipulated colonies under similar laboratory conditions (Holbrook et al. in press), indicating that the current results are not artifacts of disturbance. However, the relationship between colony size and task allocation was weaker among unmanipulated colonies, perhaps due to other, confounding sources of between-colony variation.

It is unclear why changes in colony size alone affect the proportional allocation of workers across tasks. According to the response threshold model, shifts in task allocation are driven by individual responses to changes in local, task-specific stimuli (Beshers and Fewell 2001). But we controlled the social and physical environment, other than colony size; for example, the brood-to-worker ratio was held constant and food was unlimited. Therefore, the observed differences in brood care and food processing cannot easily be explained in terms of simple stimulus-response relationships. One component of brood care, feeding larvae, is preceded by food processing; however, the two tasks changed in opposite directions, suggesting that they are not directly linked. The stimulus for waste management may have been increased by the higher rate of food processing in large colonies, which likely generated more refuse per worker.

In functionally integrated, eusocial colonies, however, workers' task decisions are not based entirely on independent responses to the environment, but can be coordinated through communication and social feedback (Hölldobler and Wilson 1990, 2009; Seeley 1995; Gordon 1996). Moreover, colony needs or task priorities may depend, in part, on the colony state, independent of external conditions (Schmid-Hempel et al. 1993; Tschinkel 2006). For example, younger and/or smaller colonies may be selected to allocate relatively more effort to tasks promoting growth, if increased size enhances colony survival and reproduction (Wilson 1971; Kolmes and Winston 1988; Schmid-Hempel et al. 1993; Kaspari and Vargo 1995; Holway and Case 2001). This could explain why proportional allocation to brood care is inversely related to colony size among size-manipulated colonies and during colony ontogeny in *P. californicus* (Holbrook et al. in press).

Consistent behavioral differences

While workers flexibly shifted their task performance in response to changes in colony size, they also exhibited consistent within-colony differences. The rank orders of brood care and waste management workers, in particular, were maintained across colony sizes, and individuals that spent more time outside of the nest in small colonies also ventured outside more often in large colonies. This behavioral consistency indicates that colony members vary intrinsically in task propensity. Such variation is a prerequisite for the response threshold model and is known to occur in many insect societies (reviewed by Robinson 1992; Beshers and Fewell 2001). When colonies contain multiple queens (polygyny), and/or queens mate with multiple males (polyandry), the resulting genotypic diversity can contribute to behavioral variability (reviewed by Oldroyd and Fewell 2007; Smith et al. 2008). Our source colonies were polygynous (two queens each), and queens of *P. californicus* are polyandrous (R.P. Overson, personal communication); we are further exploring whether this mix of matrilines and patrilines facilitates division of labor.

We verified another source of interindividual behavioral variation: worker age. Almost all eusocial insects exhibit some form of age-based division of labor, or temporal polyethism; as workers mature, they typically progress from performing inside-nest tasks such as brood care to performing outside-nest tasks such as foraging (Oster and Wilson 1978; Hölldobler and Wilson 1990; Traniello and Rosengaus 1997). Individual behavioral development is accompanied by physiological changes, which may modulate workers' response thresholds for various tasks (reviewed by Robinson 1992; Robinson 2009). Although we only crudely estimated worker age from cuticle pigmentation, we found clear differences in task performance and spatial tendency that meet the general expectation; compared to mature workers, callow workers were more likely to perform brood care and less likely to forage, perform waste management, or exit the nest. Furthermore, our results are consistent with the pattern of temporal repertoire expansion described in the ant *Pheidole dentata* (Seid and Traniello 2006); older *P. californicus* workers have larger behavioral repertoires that include inside- and outside-nest tasks.

Consistent between-individual differences in behavior expressed across contexts or situations, such as colony size, are recognized in other taxa as ‘personalities’ (Gosling 2001; Réale et al. 2007) or ‘behavioral syndromes’ (Sih et al. 2004a; Sih et al. 2004b). However, there has been little exchange between those emerging frameworks and research on interindividual behavioral variability in insect societies, despite broad conceptual and technical overlap (but see Rueppell et al. 2006; Barth et al. 2010; Muller et al. 2010; IUSSI 2010 Symposium on 'Behavioral syndromes in social insects'). Perhaps both traditions stand to benefit from integrating approaches to common questions, such as, what are the proximate mechanisms underlying behavioral differences, (how) are multiple behaviors or response thresholds correlated within individuals, are there phenotypic tradeoffs between consistency (specialization) and plasticity (flexibility), and what are the ecological and evolutionary implications of between-individual (and -colony) behavioral differences in various social contexts?

Table 5.1. Effects of colony size on colony-level task allocation and worker location in *P. californicus*.

	Proportion of observations			
	Small colonies	Large colonies	$F_{1,9}$	*P*
Allogrooming	0.034 ± 0.002	0.032 ± 0.003	0.27	0.61
Brood care	0.122 ± 0.007	0.100 ± 0.009	12.45	0.006*
Food processing	0.184 ± 0.013	0.232 ± 0.008	12.13	0.007*
Foraging	0.008 ± 0.001	0.007 ± 0.001	1.22	0.30
Waste management	0.017 ± 0.003	0.036 ± 0.004	15.15	0.004*
Total task performance	0.365 ± 0.013	0.407 ± 0.012	8.27	0.02*
Outside of nest	0.230 ± 0.012	0.231 ± 0.016	0.008	0.93

Values are mean ± SE proportion of total observations per colony. 'Total task performance' is sum of five tasks. *F* is from rmANOVA (with colonies as subjects). False discovery rate B-Y adjusted $\alpha = 0.02$ (* denotes significance).

Table 5.2. Between-individual consistency in task performance and location across colony size treatments.

		Spearman rank correlation coefficients (r_s)						
Colony	*n*	Allogrooming	Brood care	Food processing	Foraging	Waste management	Total tasks	Outside of nest
A	30	0.34	0.79***	0.03	0.61**	0.62**	0.61**	0.82***
B	34	0.05	0.62***	0.14	0.28	0.62***	0.54**	0.84***
C	31	0.19	0.90***	-0.19	0.53*	0.43	0.80***	0.95***
D	29	0.25	0.83***	0.13	0.53*	0.60**	0.70***	0.83***
E	35	0.36	0.66***	0.46*	0.15	0.56**	0.83***	0.88***
F	31	0.12	0.91***	0.41	0.38	0.65***	0.65***	0.84***
G	31	0.20	0.78***	0.49*	0.52*	0.48*	0.48*	0.89***
H	33	0.20	0.73***	-0.04	0.24	0.43	0.43	0.82***
I	27	0.40	0.86***	0.15	0.59**	0.59*	0.50*	0.77***
J	34	0.34	0.83***	0.00	-0.06	0.68***	0.51*	0.90***

Values are Spearman rank correlation coefficients (r_s) between the proportions of observations during which each individual was engaged in each task (or located outside of the nest) in small vs. large experimental colonies (*n* = number of observed individuals). Measures of foraging and waste management were conservative (see text); ‘Outside of nest’ is loosely associated with those tasks. False discovery rate B-Y adjusted $\alpha = 0.01$ (*$P \leq 0.01$; **$P \leq 0.001$; ***$P \leq 0.0001$).

Figure 5.1. Effects of colony size on (a) division of labor, (b-g) colony-level task allocation, and (h) worker location in *P. californicus*. Each bar represents a source colony. Y axes show differences between large and small experimental colonies in (a) $\mathrm{DOL}_{\text{indiv-tasks}}$ (the degree to which individuals specialize on a subset of tasks), or in the proportion of total worker-observations (b-g) allocated to each task or (h) during which workers were located outside of the nest. False discovery rate B-Y adjusted $\alpha = 0.02$ (*$P \leq 0.02$).

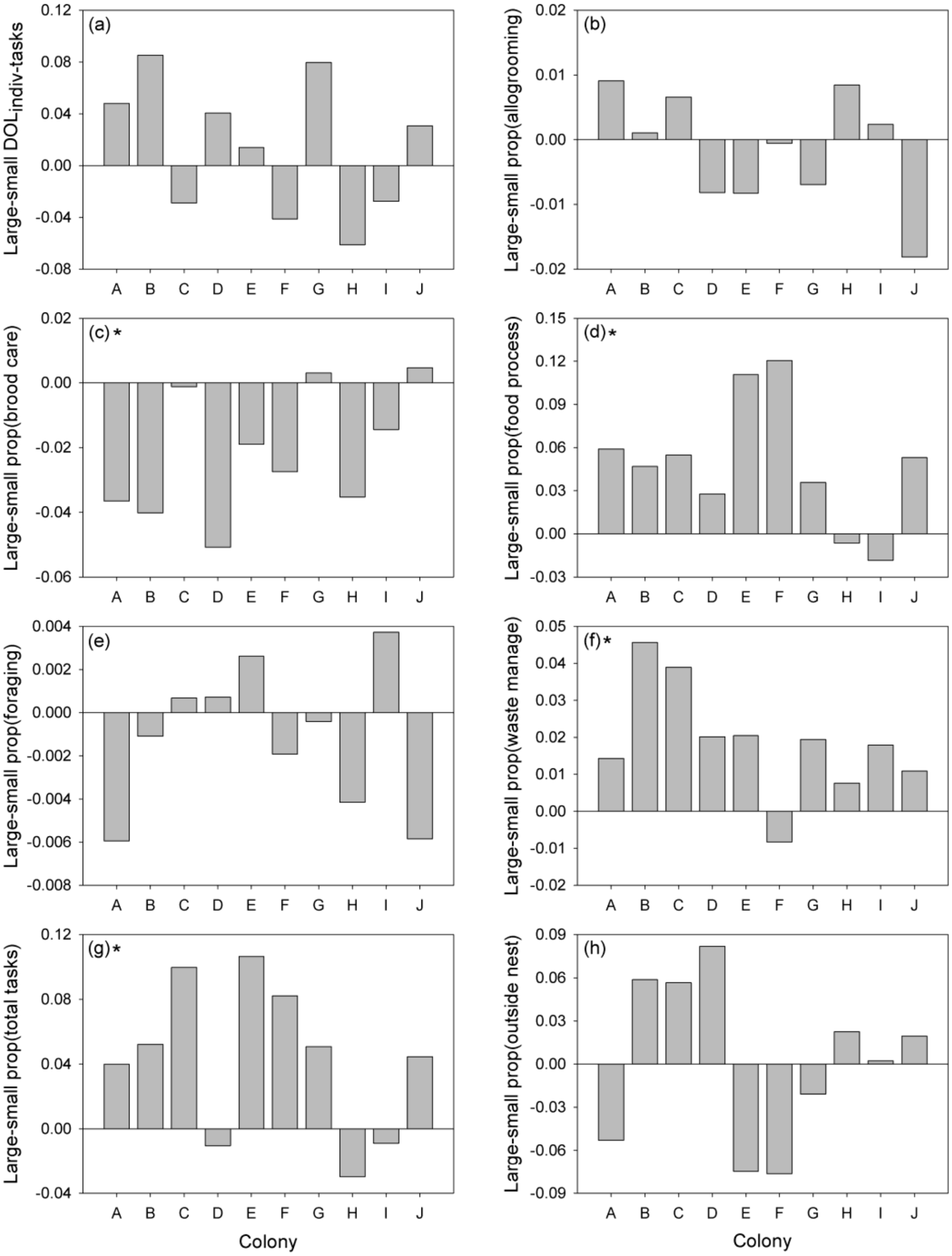

Figure 5.1, continued.

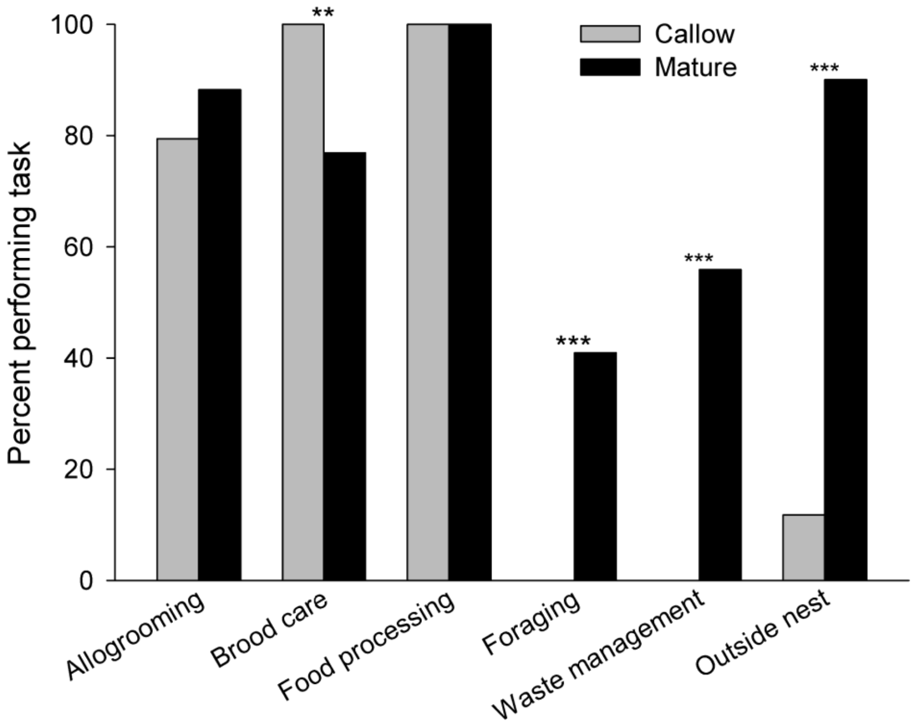

Figure 5.2. Effects of worker age on task performance and spatial tendency in *P. californicus*. Gray bars represent callow workers; black bars represent mature workers. Values are percentages of workers in each age class that performed each task or exited the nest at least once, across colony size treatments. Workers were pooled across colonies ($n_{\text{callow}} = 34$, $n_{\text{mature}} = 281$). False discovery rate B-Y adjusted $\alpha = 0.02$ (**$P = 0.002$, ***$P \leq 0.0001$).

LITERATURE CITED

Abrams, J., and G.C. Eickwort. 1981. Nest switching and guarding by the communal sweat bee *Agapostemon virescens* (Hymenoptera, Halictidae). *Insectes Sociaux* 28 (2): 105-116.

Altmann, J. 1974. Observational study of behavior: sampling methods. *Behaviour* 49 (3): 227-267.

Anderson, C., and D.W. McShea. 2001. Individual versus social complexity, with particular reference to ant colonies. *Biological Reviews* 76 (2): 211-237.

Arnold, K.E., I.P.F. Owens, and A.W. Goldizen. 2005. Division of labour within cooperatively breeding groups. *Behaviour* 142: 1577-1590.

Barth, M.B., K. Kellner, and J. Heinze. 2010. The police are not the army: context-dependent aggressiveness in a clonal ant. *Biology Letters* 6 (3): 329-332.

Barton, R.A. 1996. Neocortex size and behavioural ecology in primates. *Proceedings of the Royal Society of London B-Biological Sciences* 263 (1367): 173-177.

Bednarz, J.C. 1988. Cooperative hunting in Harris' hawks (*Parabuteo unicinctus*). *Science* 239 (4847): 1525-1527.

Bell, G., and A.O. Mooers. 1997. Size and complexity among multicellular organisms. *Biological Journal of the Linnean Society* 60 (3): 345-363.

Benjamini, Y., and D. Yekutieli. 2001. The control of the false discovery rate in multiple testing under dependency. *Annals of Statistics* 29 (4): 1165-1188.

Beshers, S.N., and J.H. Fewell. 2001. Models of division of labor in social insects. *Annual Review of Entomology* 46: 413-440.

Beshers, S.N., G.E. Robinson, and J.E. Mittenthal. 1999. Response thresholds and division of labor in insect colonies. In *Information Processing in Social Insects*, edited by C. Detrain, J. L. Deneubourg and J. M. Pasteels. Basel, Switzerland: Birkhauser Verlag.

Bonabeau, E., G. Theraulaz, and J.L. Deneubourg. 1996. Quantitative study of the fixed threshold model for the regulation of division of labour in insect societies. *Proceedings of the Royal Society of London Series B-Biological Sciences* 263 (1376): 1565-1569.

———. 1998. Fixed response thresholds and the regulation of division of labor in insect societies. *Bulletin of Mathematical Biology* 60 (4): 753-807.

Bonabeau, E., G. Theraulaz, J.L. Deneubourg, S. Aron, and S. Camazine. 1997. Self-organization in social insects. *Trends in Ecology & Evolution* 12 (5): 188-193.

Bonner, J.T. 2004. Perspective: the size-complexity rule. *Evolution* 58 (9): 1883-1890.

Bourke, A.F.G. 1999. Colony size, social complexity and reproductive conflict in social insects. *Journal of Evolutionary Biology* 12 (2): 245-257.

Breed, M.D., J.M. Silverman, and W.J. Bell. 1978. Agonistic behavior, social interactions, and behavioral specialization in a primitively eusocial bee. *Insectes Sociaux* 25 (4): 351-364.

Brown, J.H., and G.B. West, eds. 2000. *Scaling in Biology*. New York, NY: Oxford University Press.

Brown, J.J., and J.F.A. Traniello. 1998. Regulation of brood-care behavior in the dimorphic castes of the ant *Pheidole morrisi* (Hymenoptera: Formicidae): effects of caste ratio, colony size, and colony needs. *Journal of Insect Behavior* 11 (2): 209-219.

Bruintjes, R., and M. Taborsky. 2010. Size-dependent task specialization in a cooperative cichlid in response to experimental variation of demand. *Animal Behaviour* 81 (2): 387-394.

Calabi, P., and J.F.A. Traniello. 1989. Behavioral flexibility in age castes of the ant *Pheidole dentata*. *Journal of Insect Behavior* 2 (5): 663-677.

Camazine, S., J.L. Deneubourg, N.R. Franks, J. Sneyd, G. Theraulaz, and E. Bonabeau. 2001. *Self-organization in Biological Systems*. Princeton, NJ: Princeton University Press.

Changizi, M.A., M.A. McDannald, and D. Widders. 2002. Scaling of differentiation in networks: nervous systems, organisms, ant colonies, ecosystems, businesses, universities, cities, electronic circuits, and Legos. *Journal of Theoretical Biology* 218 (2): 215-237.

Chittka, L., and H. Muller. 2009. Learning, specialization, efficiency and task allocation in social insects. *Communicative & Integrative Biology* 2 (2): 151-154.

Chittka, L., and J.D. Thomson. 1997. Sensori-motor learning and its relevance for task specialization in bumble bees. *Behavioral Ecology and Sociobiology* 41 (6): 385-398.

Clutton-Brock, T.H., A.F. Russell, and L.L. Sharpe. 2003. Meerkat helpers do not specialize in particular activities. *Animal Behaviour* 66 (3): 531-540.

Cole, B.J. 1986. The social behavior of *Leptothorax allardycei* (Hymenoptera, Formicidae): time budgets and the evolution of worker reproduction. *Behavioral Ecology and Sociobiology* 18 (3): 165-173.

Costa, J.T. 2006. *The Other Insect Societies*. Cambridge, MA: Harvard University Press.

Costa, J.T., and K.G. Ross. 2003. Fitness effects of group merging in a social insect. *Proceedings of the Royal Society of London Series B-Biological Sciences* 270: 1697-1702.

Danforth, B.N. 1991. Female foraging and intranest behavior of a communal bee, *Perdita portalis* (Hymenoptera: Andrenidae). *Annals of the Entomological Society of America* 84 (5): 537-548.

Deneubourg, J.L., S. Goss, J.M. Pasteels, D. Fresneau, and J.P. Lachaud. 1987. Self-organization mechanisms in ant societies (II): learning in foraging and division of labor. *Experientia Supplementum* 54: 177-196.

Detrain, C., and J.M. Pasteels. 1991. Caste differences in behavioral thresholds as a basis for polyethism during food recruitment in the ant, *Pheidole pallidula* Nyl. (Hymenoptera: Myrmicinae). *Journal of Insect Behavior* 4 (2): 157-176.

———. 1992. Caste polyethism and collective defense in the ant, *Pheidole pallidula*: the outcome of quantitative differences in recruitment. *Behavioral Ecology and Sociobiology* 29 (6): 405-412.

Dial, K.P., E. Greene, and D.J. Irschick. 2008. Allometry of behavior. *Trends in Ecology & Evolution* 23 (7): 394-401.

Dornhaus, A. 2008. Specialization does not predict individual efficiency in an ant. *PLoS Biology* 6 (11): e285.

Dornhaus, A., J.A. Holley, and N.R. Franks. 2009. Larger colonies do not have more specialized workers in the ant *Temnothorax albipennis*. *Behavioral Ecology* 20 (5): 922-929.

Dornhaus, A., J.A. Holley, V.G. Pook, G. Worswick, and N.R. Franks. 2008. Why don't all workers work? Colony size and workload during emigrations in the ant *Temnothorax albipennis*. *Behav Ecol Sociobiol* 63 (1): 43-51.

Dukas, R., and P.K. Visscher. 1994. Lifetime learning by foraging honey bees. *Animal Behaviour* 48 (5): 1007-1012.

Eickwort, G.C., J.M. Eickwort, J. Gordon, M.A. Eickwort, and W.T. Wcislo. 1996. Solitary behavior in a high-altitude population of the social sweat bee *Halictus rubicundus* (Hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology* 38 (4): 227-233.

Elgar, M.A. 1989. Predator vigilance and group size in mammals and birds: a critical review of the empirical evidence. *Biological Reviews* 64 (1): 13-33.

Evans, H.E., and A.W. Hook. 1986. Nesting behavior of Australian *Cerceris* digger wasps, with special reference to nest reutilization and nest sharing (Hymenoptera, Sphecidae). *Sociobiology* 11: 275-302.

Fetherston, I.A., M.P. Scott, and J.F.A. Traniello. 1990. Parental care in burying beetles: the organization of male and female brood care behavior. *Ethology* 85 (3): 177-190.

Fewell, J.H. 2003. Social insect networks. *Science* 301: 1867-1870.

Fewell, J.H., and S.M. Bertram. 1999. Division of labor in a dynamic environment: response by honeybees (*Apis mellifera*) to graded changes in colony pollen stores. *Behavioral Ecology and Sociobiology* 46 (3): 171-179.

Fewell, J.H., and R.E. Page. 1993. Genotypic variation in foraging responses to environmental stimuli by honey bees, *Apis mellifera*. *Experientia* 49 (12): 1106-1112.

———. 1999. The emergence of division of labour in forced associations of normally solitary ant queens. *Evolutionary Ecology Research* 1 (5): 537-548.

———. 2000. Colony-level selection effects on individual and colony foraging task performance in honeybees, *Apis mellifera* L. *Behavioral Ecology and Sociobiology* 48 (3): 173-181.

Fewell, J.H., S.K. Schmidt, and T. Taylor. 2009. Division of labor in the context of complexity. In *Organization of Insect Societies: From Genome to Sociocomplexity*, edited by J. Gadau and J. H. Fewell. Cambridge, MA: Harvard University Press.

Fewell, J.H., and M.L. Winston. 1992. Colony state and regulation of pollen foraging in the honey bee, *Apis mellifera* L. *Behavioral Ecology and Sociobiology* 30 (6): 387-393.

Fjerdingstad, E.J., and R.H. Crozier. 2006. The evolution of worker caste diversity in social insects. *American Naturalist* 167 (3): 390-400.

Franks, N.R., A. Dornhaus, J.A.R. Marshall, and F.X.D. Moncharmont. 2009. The dawn of a golden age in mathematical insect sociobiology. In *Organization of Insect Societies: From Genome to Sociocomplexity*, edited by J. Gadau and J. H. Fewell. Cambridge, MA: Harvard University Press.

Franks, N.R., and C. Tofts. 1994. Foraging for work: how tasks allocate workers. *Animal Behaviour* 48 (2): 470-472.

Gadagkar, R. 1990. Evolution of eusociality: the advantage of assured fitness returns. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* 329 (1252): 17-25.

Gautrais, J., G. Theraulaz, J.L. Deneubourg, and C. Anderson. 2002. Emergent polyethism as a consequence of increased colony size in insect societies. *Journal of Theoretical Biology* 215 (3): 363-373.

Gazda, S.K., R.C. Connor, R.K. Edgar, and F. Cox. 2005. A division of labour with role specialization in group-hunting bottlenose dolphins (*Tursiops truncatus*) off Cedar Key, Florida. *Proceedings of the Royal Society of London Series B-Biological Sciences* 272 (1559): 135-140.

Geisser, S., and S.W. Greenhouse. 1958. An extension of Box's results on the use of the *F* distribution in multivariate analysis. *Annals of Mathematical Statistics* 29: 885-891.

Gordon, D.M. 1989. Dynamics of task switching in harvester ants. *Animal Behaviour* 38: 194-204.

———. 1996. The organization of work in social insect colonies. *Nature* 380 (6570): 121-124.

Gordon, D.M., R.E. Paul, and K. Thorpe. 1993. What is the function of encounter patterns in ant colonies? *Animal Behaviour* 45: 1083-1100.

Gorelick, R., S.M. Bertram, P.R. Killeen, and J.H. Fewell. 2004. Normalized mutual entropy in biology: quantifying division of labor. *American Naturalist* 164 (5): 677-682.

Gosling, S.D. 2001. From mice to men: what can we learn about personality from animal research? *Psychological Bulletin* 127 (1): 45-86.

Hamilton, A., N.R. Smith, and M.H. Haber. 2009. Social insects and the individuality thesis: cohesion and the colony as a selectable individual. In *Organization of Insect Societies: From Genome to Sociocomplexity*, edited by J. Gadau and J. H. Fewell. Cambridge, MA: Havard University Press.

Hart, A.G., and F.L.W. Ratnieks. 2001. Task partitioning, division of labour and nest compartmentalisation collectively isolate hazardous waste in the leafcutting ant *Atta cephalotes*. *Behavioral Ecology and Sociobiology* 49 (5): 387-392.

Helms Cahan, S., and J.H. Fewell. 2004. Division of labor and the evolution of task sharing in queen associations of the harvester ant *Pogonomyrmex californicus*. *Behavioral Ecology and Sociobiology* 56 (1): 9-17.

Hemelrijk, C.K. 2002. Understanding social behaviour with the help of complexity science (invited article). *Ethology* 108 (8): 655-671.

Herbers, J.M. 1981. Reliability theory and foraging by ants. *Journal of Theoretical Biology* 89: 175-189.

Hogendoorn, K., and M.P. Schwarz. 1998. Guarding specialisation in pre-reproductive colonies of the allodapine bee *Exoneura bicolor*. *Ethology Ecology & Evolution* 10 (1): 67-77.

Hogendoorn, K., and H.H.W. Velthuis. 1999. Task allocation and reproductive skew in social mass provisioning carpenter bees in relation to age and size. *Insectes Sociaux* 46 (3): 198-207.

Hogeweg, P., and B. Hesper. 1983. The ontogeny of the interaction structure in bumble bee colonies: A MIRROR model. *Behavioral Ecology and Sociobiology* 12 (4): 271-283.

Holbrook, C.T., P.M. Barden, and J.H. Fewell. in press. Division of labor increases with colony size in the harvester ant *Pogonomyrmex californicus*. *Behavioral Ecology*.

Holbrook, C.T., R.M. Clark, R. Jeanson, S.M. Bertram, P.F. Kukuk, and J.H. Fewell. 2009. Emergence and consequences of division of labor in associations of normally solitary sweat bees. *Ethology* 115 (4): 301-310.

Hölldobler, B., and E.O. Wilson. 1990. *The Ants*. Cambridge, MA: Harvard University Press.

———. 2009. *The Superorganism: The Beauty, Elegance, and Strangeness of Insect Societies*. New York, NY: W.W. Norton & Company.

Holway, D.A., and T.J. Case. 2001. Effects of colony-level variation on competitive ability in the invasive Argentine ant. *Animal Behaviour* 61 (6): 1181-1192.

Hou, C., M. Kaspari, H.B. Vander Zanden, and J.F. Gillooly. 2010. Energetic basis of colonial living in social insects. *Proceedings of the National Academy of Sciences* 107 (8): 3634-3638.

Huang, Z.Y., and G.E. Robinson. 1999. Social control of division of labor in honey bee colonies. In *Information Processing in Social Insects*, edited by C. Detrain, J. L. Deneubourg and J. M. Pasteels. Basel, Switzerland: Birkhäuser.

Jandt, J.M., and A. Dornhaus. 2009. Spatial organization and division of labour in the bumblebee *Bombus impatiens*. *Animal Behaviour* 77 (3): 641-651.

Jeanne, R.L. 1986. The organization of work in *Polybia occidentalis*: costs and benefits of specialization in a social wasp. *Behavioral Ecology and Sociobiology* 19 (5): 333-341.

———. 2003. Social complexity in the Hymenoptera, with special attention to the wasps. In *Genes, Behaviors and Evolution of Social Insects*, edited by T. Kikuchi, N. Azuma and S. Higashi. Sapporo: Hokkaido University Press.

Jeanson, R., R.M. Clark, C.T. Holbrook, S.M. Bertram, J.H. Fewell, and P.F. Kukuk. 2008. Division of labour and socially induced changes in response thresholds in associations of solitary halictine bees. *Animal Behaviour* 76 (3): 593-602.

Jeanson, R., and J.H. Fewell. 2008. Influence of the social context on division of labor in ant foundress associations. *Behavioral Ecology* 19 (3): 567-574.

Jeanson, R., J.H. Fewell, R. Gorelick, and S.M. Bertram. 2007. Emergence of increased division of labor as a function of group size. *Behavioral Ecology and Sociobiology* 62 (2): 289-298.

Jeanson, R., P.F. Kukuk, and J.H. Fewell. 2005. Emergence of division of labour in halictine bees: contributions of social interactions and behavioural variance. *Animal Behaviour* 70 (5): 1183-1193.

Johnson, B.R., and T.A. Linksvayer. 2010. Deconstructing the superorganism: social physiology, groundplans, and sociogenomics. *The Quarterly Review of Biology* 85 (1): 57-79.

Johnson, R.A. 2000a. Seed-harvester ants (Hymenoptera: Formicidae) of North America: an overview of ecology and biogeography. *Sociobiology* 36 (1): 89-122 + 83-88.

———. 2000b. Water loss in desert ants: caste variation and the effect of cuticle abrasion. *Physiological Entomology* 25 (1): 48-53.

———. 2004. Colony founding by pleometrosis in the semiclaustral seed-harvester ant *Pogonomyrmex californicus* (Hymenoptera: Formicidae). *Animal Behaviour* 68 (5): 1189-1200.

Jones, J.C., M.R. Myerscough, S. Graham, and B.P. Oldroyd. 2004. Honey bee nest thermoregulation: diversity promotes stability. *Science* 305 (5682): 402-404.

Julian, G.E., and S. Cahan. 1999. Undertaking specialization in the desert leaf-cutter ant *Acromyrmex versicolor*. *Animal Behaviour* 58 (2): 437-442.

Jun, J., J.W. Pepper, V.M. Savage, J.F. Gillooly, and J.H. Brown. 2003. Allometric scaling of ant foraging trail networks. *Evolutionary Ecology Research* 5 (2): 297-303.

Karsai, I., and J.W. Wenzel. 1998. Productivity, individual-level and colony-level flexibility, and organization of work as consequences of colony size. *Proceedings of the National Academy of Sciences of the United States of America* 95 (15): 8665-8669.

Kaspari, M., and E.L. Vargo. 1995. Colony size as a buffer against seasonality: Bergmann's rule in social insects. *American Naturalist* 145 (4): 610.

Kolmes, S.A., and M.L. Winston. 1988. Division of labour among worker honey bees in demographically manipulated colonies. *Insectes Sociaux* 35 (3): 262-270.

Kukuk, P.F. 1992. Social interactions and familiarity in a communal halictine bee *Lasioglossum (Chilalictus) hemichalceum*. *Ethology* 91 (4): 291-300.

Kukuk, P.F., S.A. Ward, and A. Jozwiak. 1998. Mutualistic benefits generate an unequal distribution of risky activities among unrelated group members. *Naturwissenschaften* 85 (9): 445-449.

Lacey, E.A., and P.W. Sherman. 1997. Cooperative breeding in naked mole-rats: Implications for vertebrate and invertebrate sociality. In *Cooperative Breeding in Mammals*, edited by N. G. Solomon and J. A. French. Cambridge, UK: Cambridge University Press.

Lin, N., and C.D. Michener. 1972. Evolution of sociality in insects. *Quarterly Review of Biology* 47 (2): 131-159.

Lindauer, M. 1961. *Communication Among Social Bees*. Cambridge, MA: Harvard University Press.

Mailleux, A.C., J.L. Deneubourg, and C. Detrain. 2003. How does colony growth influence communication in ants? *Insectes Sociaux* 50 (1): 24-31.

Maynard Smith, J., and E. Szathmáry. 1995. *The Major Transitions in Evolution*. Oxford, U.K.: Oxford University Press.

McConnell-Garner, J., and P.F. Kukuk. 1997. Behavioral interactions of two solitary, halictine bees with comparisons among solitary, communal and eusocial species. *Ethology* 103 (1): 19-32.

McCorquodale, D.B. 1989. Soil softness, nest initiation and nest sharing in the wasp, *Cerceris antipodes* (Hymenoptera: Sphecidae). *Ecological Entomology* 14: 191-196.

Merkle, D., and M. Middendorf. 2004. Dynamic polyethism and competition for tasks in threshold reinforcement models of social insects. *Adaptive Behavior* 12 (3-4): 251.

Michener, C.D. 1974. *The Social Behavior of the Bees: A Comparative Study*. Cambridge, MA: Harvard University Press.

Muller, H., H. Grossmann, and L. Chittka. 2010. 'Personality' in bumblebees: individual consistency in responses to novel colours? *Animal Behaviour* 80: 1065-1074.

Nakagawa, S. 2004. A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behavioral Ecology* 15 (6): 1044-1045.

Nakaya, F., Y. Saito, and T. Motokawa. 2003. Switching of metabolic-rate scaling between allometry and isometry in colonial ascidians. *Proceedings of the Royal Society of London B-Biological Sciences* 270 (1520): 1105-1113.

Narum, S.R. 2006. Beyond Bonferroni: less conservative analyses for conservation genetics. *Conservation Genetics* 7 (5): 783-787.

O'Donnell, S. 1998. Dominance and polyethism in the eusocial wasp *Mischocyttarus mastigophorus* (Hymenoptera: Vespidae). *Behavioral Ecology and Sociobiology* 43 (4): 327-331.

———. 2001. Worker biting interactions and task performance in a swarm-founding eusocial wasp (*Polybia occidentalis*, Hymenoptera: Vespidae). *Behavioral Ecology* 12 (3): 353-359.

O'Donnell, S., and R.L. Foster. 2001. Thresholds of response in nest thermoregulation by worker bumble bees, *Bombus bifarius nearcticus* (Hymenoptera: Apidae). *Ethology* 107 (5): 387-399.

Oldroyd, B.P., and J.H. Fewell. 2007. Genetic diversity promotes homeostasis in insect colonies. *Trends in Ecology & Evolution* 22 (8): 408-413.

Oster, G.F., and E.O. Wilson. 1978. *Caste and Ecology in the Social Insects*. Princeton, NJ: Princeton University Press.

Pacala, S.W., D.M. Gordon, and H.C.J. Godfray. 1996. Effects of social group size on information transfer and task allocation. *Evolutionary Ecology* 10 (2): 127-165.

Packer, L. 2006. Use of artificial arenas to predict the social organisation of halictine bees: data for fourteen species from Chile. *Insectes Sociaux* 53 (3): 307-315.

Page, R.E. 1997. The evolution of insect societies. *Endeavour* 21 (3): 114-120.

Page, R.E., and S.D. Mitchell. 1998. Self-organization and the evolution of division of labor. *Apidologie* 29 (1-2): 171-190.

Peters, R.H. 1983. *The Ecological Implications of Body Size*. Cambridge, UK: Cambridge University Press.

Pielou, E.C. 1966. The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology* 13: 131-144.

Plowright, R.C., and C.M.S. Plowright. 1988. Elitism in social insects: a positive feedback model. In *Interindividual Behavioral Variability in Social Insects*, edited by R. L. Jeanne. Boulder, CO: Westview Press.

Pollard, K.A., and D.T. Blumstein. 2008. Time allocation and the evolution of group size. *Animal Behaviour* 76 (5): 1683-1699.

———. 2011. Social group size predicts the evolution of individuality. *Current Biology* 21 (5): 1-5.

Porter, S.D., and W.R. Tschinkel. 1985. Fire ant polymorphism: the ergonomics of brood production. *Behavioral Ecology and Sociobiology* 16 (4): 323-336.

Powell, S., and W.R. Tschinkel. 1999. Ritualized conflict in *Odontomachus brunneus* and the generation of interaction-based task allocation: a new organizational mechanism in ants. *Animal Behaviour* 58 (5): 965-972.

Pratt, S.C. 2005. Quorum sensing by encounter rates in the ant *Temnothorax albipennis*. *Behavioral Ecology* 16 (2): 488-496.

Queller, D.C. 1993. Worker control of sex-ratios and selection for extreme multiple mating by queens. *American Naturalist* 142 (2): 346-351.

———. 1994. Extended parental care and the origin of eusociality. *Proceedings of the Royal Society of London Series B-Biological Sciences* 256 (1346): 105-111.

Queller, D.C., and J.E. Strassmann. 2009. Beyond society: the evolution of organismality. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364 (1533): 3143-3155.

Ravary, F., E. Lecoutey, G. Kaminski, N. Châline, and P. Jaisson. 2007. Individual experience alone can generate lasting division of labor in ants. *Current Biology* 17 (15): 1308-1312.

Réale, D., S.M. Reader, D. Sol, P.T. McDougall, and N.J. Dingemanse. 2007. Integrating animal temperament within ecology and evolution. *Biological Reviews* 82 (2): 291-318.

Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* 43 (1): 223-225.

Ridley, A.R., and N.J. Raihani. 2008. Task partitioning increases reproductive output in a cooperative bird. *Behavioral Ecology* 19 (6): 1136-1142.

Robinson, E. 2009. Physiology as a caste-defining feature. *Insectes Sociaux* 56 (1): 1-6.

Robinson, E.J.H., O. Feinerman, and N.R. Franks. 2009. Flexible task allocation and the organization of work in ants. *Proceedings of the Royal Society B* 276: 4373-4380.

Robinson, G.E. 1992. Regulation of division of labor in insect societies. *Annual Review of Entomology* 37 (1): 637-665.

Robinson, G.E., and R.E. Page. 1989. Genetic basis for division of labor in an insect society. In *The Genetics of Social Evolution*, edited by M. D. Breed and R. E. Page. Boulder, CO: Westview.

Robson, S.K.A., K. Bean, J. Hansen, K. Norling, R.J. Rowe, and D. White. 2000. Social and spatial organisation in colonies of a primitively eusocial wasp, *Ropalidia revolutionalis* (de Saussure) (Hymenoptera: Vespidae). *Australian Journal of Entomology* 39 (1): 20-24.

Rueppell, O., S.B.C. Chandra, T. Pankiw, M.K. Fondrk, M. Beye, G. Hunt, and R.E. Page. 2006. The genetic architecture of sucrose responsiveness in the honeybee (*Apis mellifera* L.). *Genetics* 172 (1): 243-251.

Sakagami, S.F., and Y. Maeta. 1987. Sociality, induced and/or natural, in the basically solitary small carpenter bees (*Ceratina*). In *Animal Societies: Theories and Facts*, edited by Y. Ito, J. L. Brown and J. Kikkawa. Tokyo: Japan Scientific Societies Press.

Schmid-Hempel, P. 1990. Reproductive competition and the evolution of work load in social insects. *American Naturalist* 135 (4): 501.

Schmid-Hempel, P., M.L. Winston, and R.C. Ydenberg. 1993. Foraging of individual workers in relation to colony state in the social Hymenoptera. *Canadian Entomologist* 125: 129-160.

Schmidt-Nielsen, K. 1984. *Scaling: Why is Animal Size So Important?* Cambridge, UK: Cambridge University Press.

Schwander, T., H. Rosset, and M. Chapuisat. 2005. Division of labour and worker size polymorphism in ant colonies: the impact of social and genetic factors. *Behavioral Ecology and Sociobiology* 59 (2): 215-221.

Schwarz, M.P., M.H. Richards, and B.N. Danforth. 2007. Changing paradigms in insect social evolution: insights from halictine and allodapine bees. *Annual Review of Entomology* 52: 127–150.

Seeley, T.D. 1982. Adaptive significance of the age polyethism schedule in honeybee colonies. *Behavioral Ecology and Sociobiology* 11 (4): 287-293.

———. 1995. *The Wisdom of the Hive: The Social Physiology of Honey Bee Colonies*. Cambridge, MA: Harvard University Press.

———. 1997. Honey bee colonies are group-level adaptive units. *American Naturalist* 150 (S1): 22-41.

Seid, M.A., and J.F.A. Traniello. 2006. Age-related repertoire expansion and division of labor in *Pheidole dentata* (Hymenoptera: Formicidae): a new perspective on temporal polyethism and behavioral plasticity in ants. *Behavioral Ecology and Sociobiology* 60 (5): 631-644.

Sendova-Franks, A.B., and N.R. Franks. 1994. Social resilience in individual worker ants and its role in division of labour. *Proceedings of the Royal Society of London B-Biological Sciences* 256 (1347): 305-309.

———. 1995. Spatial relationships within nests of the ant *Leptothorax unifasciatus* (Latr.) and their implications for the division of labour. *Animal Behaviour* 50 (1): 121-136.

Shannon, C.E. 1948. A mathematical theory of communication. *Bell System Technical Journal* 27: 379–423, 623-656.

Sih, A., A. Bell, and J.C. Johnson. 2004a. Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution* 19 (7): 372-378.

Sih, A., A.M. Bell, J.C. Johnson, and R.E. Ziemba. 2004b. Behavioral syndromes: an integrative overview. *Quarterly Review of Biology* 79 (3): 241-277.

Smith, A. 1776 [1976]. *The Nature and Causes of the Wealth of Nations*. Chicago, IL: University of Chicago Press.

Smith, A.R., W.T. Wcislo, and S. O'Donnell. 2003. Assured fitness returns favor sociality in a mass-provisioning sweat bee, *Megalopta genalis* (Hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology* 54 (1): 14-21.

Smith, C., A. Toth, A. Suarez, and G. Robinson. 2008. Genetic and genomic analyses of the division of labour in insect societies. *Nature Reviews Genetics* 9 (10): 735-748.

Sokal, R.R., and F.J. Rohlf. 1995. *Biometry*. 3rd ed. New York: W.H. Freeman and Co.

Spessa, A., M.P. Schwarz, and M. Adams. 2000. Sociality in *Amphylaeus morosus* (Hymenoptera: Colletidae: Hylaeinae). *Annals of the Entomological Society of America* 93 (3): 684-692.

Stander, P.E. 1992. Cooperative hunting in lions: the role of the individual. *Behavioral Ecology and Sociobiology* 29 (6): 445-454.

Sumpter, D.J.T. 2006. The principles of collective animal behaviour. *Philosophical Transactions of the Royal Society B-Biological Sciences* 361 (1465): 5-22.

Theraulaz, G., E. Bonabeau, and J.L. Deneubourg. 1998. Response threshold reinforcement and division of labour in insect societies. *Proceedings of the Royal Society of London Series B-Biological Sciences* 265 (1393): 327-332.

Thomas, M.L., and M.A. Elgar. 2003. Colony size affects division of labour in the ponerine ant *Rhytidoponera metallica*. *Naturwissenschaften* 90 (2): 88-92.

Tofts, C., and N.R. Franks. 1992. Doing the right thing: ants, honeybees and naked mole-rats. *Trends in Ecology & Evolution* 7 (10): 346-349.

Traniello, J.F.A., and R.B. Rosengaus. 1997. Ecology, evolution and division of labour in social insects. *Animal Behaviour* 53 (1): 209-213.

Trumbo, S.T., and G.E. Robinson. 1997. Learning and task interference by corpse-removal specialists in honey bee colonies. *Ethology* 103 (11): 966-975.

Tschinkel, W.R. 1988. Colony growth and the ontogeny of worker polymorphism in the fire ant, *Solenopsis invicta*. *Behavioral Ecology and Sociobiology* 22 (2): 103-115.

———. 1993. Sociometry and sociogenesis of colonies of the fire ant *Solenopsis invicta* during one annual cycle. *Ecological Monographs* 63 (4): 425-457.

———. 2004. The nest architecture of the Florida harvester ant, *Pogonomyrmex badius*. *Journal of Insect Science* 4 (21).

———. 2006. *The Fire Ants*. Cambridge, MA: The Belknap Press of Harvard University Press.

Underwood, D.L.A., and A.M. Shapiro. 1999. Evidence for division of labor in the social caterpillar *Eucheira socialis* (Lepidoptera: Pieridae). *Behavioral Ecology and Sociobiology* 46 (4): 228-236.

von Ende, C.N. 1993. Repeated-measures analysis: growth and other time-dependent measures. In *Design and Analysis of Ecological Experiments*, edited by S. M. Scheiner and J. Gurevitch. New York, NY: Chapman & Hall.

Waters, J.W., C.T. Holbrook, J.H. Fewell, and J.F. Harrison. 2010. Allometric scaling of metabolism, growth, and activity in whole colonies of the seed-harvester ant *Pogonomyrmex californicus*. *American Naturalist* 176: 501-510.

Wcislo, W.T. 1997a. Behavioral experiments of sweat bees (Halictinae) in relation to variability in social organization. In *The Evolution of Social Behavior in Insects and Arachnids*, edited by J. C. Choe and B. J. Crespi. Cambridge, UK: Cambridge University Press.

———. 1997b. Social interactions and behavioral context in a largely solitary bee, *Lasioglossum (Dialictus) figueresi* (Hymenoptera, Halictidae). *Insectes Sociaux* 44 (3): 199-208.

Wcislo, W.T., and S.M. Tierney. 2009. The evolution of communal behavior in bees and wasps: an alternative to eusociality. In *Organization of Insect Societies: From Genome to Sociocomplexity*, edited by J. Gadau and J. H. Fewell. Cambridge, MA: Harvard University Press.

Wcislo, W.T., A. Wille, and E. Orozco. 1993. Nesting biology of tropical solitary and social sweat bees, *Lasioglossum (Dialictus) figueresi* Wcislo and *L. (D.) aeneiventre* (Friese) (Hymenoptera: Halictidae). *Insectes Sociaux* 40 (1): 21-40.

Weidenmuller, A. 2004. The control of nest climate in bumblebee (*Bombus terrestris*) colonies: interindividual variability and self reinforcement in fanning response. *Behavioral Ecology* 15 (1): 120-128.

Wheeler, D.E., and H.F. Nijhout. 1984. Soldier determination in *Pheidole bicarinata*: inhibition by adult soldiers. *Journal of Insect Physiology* 30 (2): 127-135.

Wheeler, W.M. 1911. The ant-colony as an organism. *Journal of Morphology* 22 (2): 307-325.

Wilson, E.O. 1971. *The Insect Societies*. Cambridge, MA: Harvard University Press.

———. 1980. Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*): II. The ergonomic optimization of leaf cutting. *Behavioral Ecology and Sociobiology* 7 (2): 157-165.

———. 1983. Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*). IV. Colony ontogeny of *A. cephalotes*. *Behavioral Ecology and Sociobiology* 14 (1): 55-60.

———. 1985. The sociogenesis of insect colonies. *Science* 228 (4707): 1489-1495.

Wilson, E.O., and B. Hölldobler. 1988. Dense heterarchies and mass communication as the basis of organization in ant colonies. *Trends in Ecology & Evolution* 3 (3): 65-68.

Winston, M.L., and L.A. Fergusson. 1985. The effect of worker loss on temporal caste structure in colonies of the honeybee (*Apis mellifera* L.). *Canadian Journal of Zoology* 63 (4): 777-780.

Yip, E.C., K.S. Powers, and L. Avilés. 2008. Cooperative capture of large prey solves scaling challenge faced by spider societies. *Proceedings of the National Academy of Sciences* 105 (33): 11818-11822.

Young, A.A. 1928. Increasing returns and economic progress. *The Economic Journal* 38: 527-542.

Zar, J.H. 1999. *Biostatistical Analysis: Fourth Edition*. Upper Saddle River, NJ: Prentice Hall.

APPENDIX A

PERMISSION TO USE PUBLISHED ARTICLES

Chapter 2 was previously published as:

> Holbrook, C.T., R.M. Clark, R. Jeanson, S.M. Bertram, P.F. Kukuk, and J.H. Fewell. 2009. Emergence and consequences of division of labor in associations of normally solitary sweat bees. *Ethology* 115 (4): 301-310.

Chapter 4 has been accepted for publication as:

> Holbrook, C.T., P.M. Barden, and J.H. Fewell. in press. Division of labor increases with colony size in the harvester ant *Pogonomyrmex californicus*. *Behavioral Ecology*.

All co-authors have granted permission to use these articles in this dissertation.