Insectes Sociaux

RESEARCH ARTICLE

Colony-size effects on task organization in the harvester ant *Pogonomyrmex californicus*

C. T. Holbrook · T. H. Eriksson · R. P. Overson · J. Gadau · J. H. Fewell

Abstract Colony size is a fundamental attribute of insect societies that appears to play an important role in their organization of work. In the harvester ant *Pogonomyrmex californicus*, division of labor increases with colony size during colony ontogeny and among unmanipulated colonies of the same age. However, the mechanism(s) integrating individual task specialization and colony size is unknown. To test whether the scaling of division of labor is an emergent epiphenomenon, as predicted by self-organizational models of task performance, we manipulated colony size in *P. californicus* and quantified short-term behavioral responses of individuals and colonies. Variation in colony size failed to elicit a change in division of labor, suggesting that colony-size effects on task specialization are mediated by slower developmental processes and/or correlates of colony size that were missing from our experiment. In contrast, the proportional allocation of workers to tasks shifted with colony size, suggesting that task needs or 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. Sources of interindividual behavioral variability include worker age and genotype (matriline).

Introduction

The outstanding ecological success of eusocial insects can be partly attributed to their efficient organization of work (Oster and Wilson, 1978). A prominent pattern of colony 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 task specialization with the flexibility to reallocate workers in response to changes in task demand, which depends on variable internal and external conditions (Calabi and Traniello, 1989; Seeley, 1995; Gordon, 1996). This study aims to elucidate how individual task decisions are integrated with a fundamental colony attribute: colony size.

In eusocial insect colonies, interindividual variation in task performance is 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., 2008a; 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

C. T. Holbrook · T. H. Eriksson · R. P. Overson ·
J. Gadau · J. H. Fewell
School of Life Sciences and Center for Social Dynamics
and Complexity, Arizona State University,
Tempe, AZ 85287, USA

C. T. Holbrook (✉)
Department of Biology, Lynchburg College, 1501 Lakeside
Drive, Lynchburg, VA 24501, USA
e-mail: ctholbrook@gmail.com

different tasks, and if the performance of a task reduces the intensity of an associated stimulus, then a division of labor can emerge (self-organize) in which workers with the lowest thresholds for a given task become specialists for that task. The response threshold model also offers an explanation for how workers can be allocated to meet colony needs without central control. 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 in colonies of ants and bees (Detrain and Pasteels, 1991, 1992; Fewell and Page, 1993; O'Donnell and Foster, 2001; Weidenmuller, 2004).

Worker behavior may also be modulated by colony state parameters such as 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). Variation in colony size, which typically increases during colony ontogeny and can be abruptly diminished by predation, disturbance, disease, or reproductive events (Wilson, 1971), has been theoretically and empirically associated with patterns of task allocation (Pacala et al., 1996; Anderson and McShea, 2001; Dornhaus et al., 2012); in particular, the degree to which workers specialize on tasks is positively correlated with colony size and/or age within several species of ants and wasps (Jeanne, 1986; Gordon, 1989; Thomas and Elgar, 2003; Holbrook et al., 2011; but see Dornhaus et al., 2009). Yet, the mechanism(s) of integration between individual task specialization and colony size remains unknown.

The scaling of division of labor may simply be an epiphenomenon of self-organized task performance. Simulations of a basic response threshold model, in which individual thresholds are fixed over time, suggest that division of labor can increase automatically as an emergent consequence of increased colony size (Jeanson et al., 2007). This 'null' hypothesis is consistent with findings from artificially established groups of normally solitary ant foundresses (Jeanson and Fewell, 2008) and sweat bees (Holbrook, 2011). In more complex, functionally integrated colonies, however, colony-size effects on task specialization and division of labor may be mediated by slower developmental processes. For example, self-reinforcement of response thresholds could cause workers in larger colonies to gradually become more specialized over time (Theraulaz et al., 1998; Gautrais et al., 2002; Merkle and Middendorf, 2004). Or, if the scaling of division of labor is an evolved response (Bonner, 2004; Holbrook et al., 2011), it may be adaptively regulated by colony-level control mechanisms that are tuned to colony size (Wilson, 1985; Beshers et al., 1999). Moreover, task needs may covary with colony size, triggering shifts in the proportional allocation of workers to tasks and possibly altering the opportunity for task specialization (Schmid-Hempel et al., 1993; Thomas and Elgar, 2003; Tschinkel, 2006; Holbrook et al., 2011).

In undisturbed colonies of the harvester ant *Pogonomyrmex californicus*, division of labor scales positively with colony size during colony ontogeny and independently of colony age (Holbrook et al., 2011). To distinguish among potential proximate causes, we experimentally manipulated colony size in *P. californicus* while controlling for other sources of between-colony variation. Specifically, we asked whether a scaling relationship can emerge from short-term interactions among flexible workers (Jeanson et al., 2007), as opposed to requiring longer-term size differentiation and ensuing developmental changes. We observed the same workers in both small and large colonies so that colony-level responses could be explained in terms of underlying individual behavior. We simultaneously tested for effects of worker age and genotype (matriline), two intrinsic factors known to influence task performance in social insects (Hölldobler and Wilson, 1990; Oldroyd and Fewell, 2007; Smith et al., 2008a).

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, 1,136 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 frozen crickets or mealworms. At the start of the experiment, source colonies were 21 months old and contained two queens and between 355 and 835 monomorphic workers each (average $\pm$ SD = 599 $\pm$ 147 workers).

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 span of several days. Ten source colonies were subjected to sequential size manipulations of 50 workers ('small colony') versus 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.).

Two days before a colony was tested, we individually marked 36 workers 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 haphazardly selected from a holding container with care taken to avoid bias (e.g., body size, activity). Callow workers were approximately 3–10 days old; mature workers had lived from 2 weeks up to several months. One hour after marking workers, we established an experimental colony with one queen, either 50 or 300 workers including all marked workers, and brood, all drawn from the same source colony. 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 haphazardly selected from holding containers. To standardize nestmate density (0.4 workers/cm^2), small and large experimental colonies were housed in different-sized, plastic observation nests (small = 11 × 11 × 3.5 cm; large = 31 × 23 × 10 cm; ants could not climb walls). Each nest opened, via a 0.6-cm diameter entrance, into a 55 × 36-cm foraging arena provisioned with water (in a test tube plugged with cotton) and ad libitum Kentucky bluegrass seeds. Nest containers were lined with plaster and enclosed to retain moisture, while foraging arenas were open to the air. Foraging arena size was constant across treatments, minimizing bias in outside-nest behavioral observations (e.g., foragers travelled the same distance in small vs. large colonies and were equally likely to be observed), but not controlling for worker density or frequency of worker–worker interactions. At the beginning of each day of observation, seeds were supplemented with ~1 frozen fruit fly per ten workers to stimulate foraging. Observation nests were maintained at 28–30 °C under ambient light.

Behavioral observations were initiated after an approximately 36-h acclimation period; colonies qualitatively resumed pre-disturbance behavior within several hours. We conducted 50 scan samples per colony, separated by at least 20-min intervals, between 0700 and 1900 h for 2 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 identified; after this initial sweep, we searched for specific individuals that had been overlooked. A minority of individuals was not found during all 50 scan samples, but each worker was observed at least 41 times (average = 49.7 observations per individual).

Behavioral acts were grouped into five major tasks, following Holbrook et al. (2011):

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.

Immediately following the observation period, unmarked workers and brood from the experimental colony were recombined with the source colony, and a smaller (50 workers) or larger (300 workers) experimental colony was formed using the same marked workers and queen and following the procedure described above. New experimental colonies were allowed to acclimate for approximately 36 h before we conducted another set of 50 scan samples across 2 days. At the end of the experiment, workers and queens were preserved in 95 % EtOH and stored at −80 °C.

Genotyping

To test for effects of matriline (two per colony) on task performance, genomic DNA was obtained from focal workers and queens using a modified Chelex® (Bio-Rad, Inc., Hercules, CA, USA) extraction protocol. With the gaster removed, individuals were homogenized in 150 μl of 5 % Chelex® solution in TE pH 8.0 and 1 μl of Proteinase K in 1.7 ml Eppendorf tubes. Samples were incubated in a 57 °C water bath for 1–24 h, heated at 95 °C for 5 min, and vortexed at 14,000 rpm for 10 min, after which the supernatants were transferred to new tubes. Supernatant containing template DNA was stored at −15 °C.

We used four microsatellite primer sets to assign workers to matrilines: Pb5, Pb6 (Volny and Gordon, 2002), Ppro2 (Pol et al., 2008), and BJ04 (Gadau et al., unpubl.). Loci were amplified in a 12-μl reaction volume containing 6.4 μl of ultrapure water, 2.5 μl of 5× Go *Taq* buffer (with 1.5 mM MgCl), 0.5 μl of dNTPs, 0.5 μl of 50 mM MgCl,

0.5 μl of 10× forward primer, 0.5 μl of 10× reverse primer, 0.125 μl of *Taq* polymerase, and 1 μl of DNA template. The following PCR profile was employed using Eppendorf thermocyclers: an initial 95 °C for 5 min, followed by 30 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, and finally 72 °C for 10 min. The products were then diluted to 10× with ultrapure water and their lengths were measured on a 4300 DNA Analyzer (LI-COR, Inc., Lincoln, NE, USA). We scored alleles using Saga Generation 2 software (LI-COR) and assigned individuals to matrilines using queens' and workers' multilocus genotypes. Due to shared queen alleles in colony C, only four workers could be assigned to a matriline; in the remaining nine colonies, we were able to unambiguously deduce the matrilines of between 23 and 35 workers per colony.

Morphometrics

To test for an association between matriline and worker body size, we measured head width, a standard estimate of body size in ants. Heads were removed and photographed in full frontal view using a SPOT Insight QE digital camera mounted on a Leica MZ 125 stereomicroscope. We then used the program ImageJ (National Institutes of Health; http://rsb.info.nih.gov/ij/) to measure maximum head width (excluding eyes) from the images, which included a 1-mm scale for calibration. Head width was normally distributed within colonies (confirmed by normal probability plots).

Data analysis

Division of labor was quantified using the $DOL_{indiv\text{-}tasks}$ 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; Holbrook et al., 2011). 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. $DOL_{indiv\text{-}tasks}$ was calculated as mutual entropy between individuals and tasks ($I_{indiv,tasks}$) divided by Shannon's index or marginal entropy of tasks (H_{tasks}). For details, see Gorelick et al. (2004) and Holbrook et al. (2011). Because our direct measurement of foraging and waste management was conservative (see above), and those tasks occurred predominantly in the foraging arena, we also calculated a $DOL_{indiv\text{-}loc}$ index using locations (inside vs. outside of nest) instead of tasks.

We tested for an effect of colony size on each DOL_{indiv} index (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 that 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 determine whether individuals differed consistently 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. We then tested for effects of worker age and genotype (matriline). First, we performed Chi-squared tests to determine whether worker age (callow vs. mature) was associated with likelihood of performing each task or exiting the nest at least once (2 × 2 contingency tables); because the sample size of callow workers per colony was small, individuals were pooled across colonies ($n_{callow} = 34$, $n_{mature} = 281$). Next, we tested for within-colony differences between matrilines in their overall distributions of task performance using *G* tests (log-likelihood ratio; 2 × 5 contingency tables), based on the total number of observed task performances by mature workers (callows were excluded to control for confounding effects of worker age) under each colony size treatment and pooled across colony sizes; we excluded colonies C (only four workers assigned to any matriline) and G (one matriline represented by only one worker). To examine differences in each task separately, we subdivided contingency tables by collapsing all but one task into a single column and performed additional *G* tests. Finally, we tested for effects of matriline on total task performance and head width using nested ANOVAs, with matriline nested within colony.

Within each series of related tests, we controlled for the false discovery rate following the Benjamini–Yekutieli (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 $DOL_{indiv-tasks}$, the degree to which individuals specialized on tasks (rmANOVA: $F_{1,9} = 0.73$, $P = 0.41$), or on $DOL_{indiv-loc}$, the degree to which individuals exhibited spatial fidelity inside versus outside of the nest ($F_{1,9} = 0.19$, $P = 0.67$). $DOL_{indiv-tasks}$ varied from 0.24 to 0.36, falling within the range of values previously observed in unmanipulated, 1-year-old *P. californicus* colonies of 30–390 workers (Holbrook et al., 2011).

Colony-level task allocation, however, shifted in response to experimental colony size variation (Table 1). 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 $\alpha = 0.02$).

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: $F_{1,149} = 17.29$, $P < 0.001$; waste management: $F_{1,71} = 15.29$, $P < 0.001$). Food processing was performed by all but one focal individual in both small and large colonies, but individuals performed the task more frequently in large colonies ($F_{1,303} = 44.93$, $P < 0.001$).

Interindividual 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 2). In all 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 ($\chi_1^2 = 9.91$, $P = 0.002$) and less likely to forage ($\chi_1^2 = 21.92$, $P < 0.001$) or perform waste management ($\chi_1^2 = 37.87$, $P < 0.001$; B–Y adjusted $\alpha = 0.02$; Fig. 1). 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 ($\chi_1^2 = 123.69$, $P < 0.001$). Worker age was not associated with likelihood of allogrooming ($\chi_1^2 = 2.14$, $P = 0.14$) or food processing (performed by all workers). Overall, mature workers performed a larger repertoire of tasks than callow workers (Mann–Whitney U test: $U = 2151.5$, $N_{mature} = 281$, $N_{callow} = 34$, $P < 0.001$; Fig. 1). The results did not change when small and large colonies were analyzed

Table 1 Effects of colony size on colony-level task allocation and worker location in *P. californicus*

	Proportion of observations		$F_{1,9}$	P
	Small colonies	Large colonies		
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 tasks	0.365 ± 0.013	0.407 ± 0.012	8.27	0.018*
Outside of nest	0.230 ± 0.012	0.231 ± 0.016	0.008	0.93

Values are mean $\pm$ SE proportion of observations during which workers were engaged in each task or located outside of the nest ($n = 10$ colonies; 315 focal individuals; 15,741 total observations of small colonies; 15,555 total observations of large colonies). 'Total tasks' is sum of five tasks. F is from rmANOVA (with colonies as subjects). False discovery rate B–Y adjusted $\alpha = 0.019$ (7 tests; * denotes significance)

Table 2 Between-individual consistency in task performance and location across colony size treatments

Colony	n	Spearman rank correlation coefficient (r_s)						
		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***	0.002	−0.06	0.68***	0.51**	0.90***

Spearman rank correlations were calculated between proportions of observations during which each individual was engaged in each task (or located outside of the nest) in small versus large experimental colonies. 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 α = 0.019 (7 tests per colony; * $P \leq 0.019$; ** $P \leq 0.01$; *** $P \leq 0.001$)
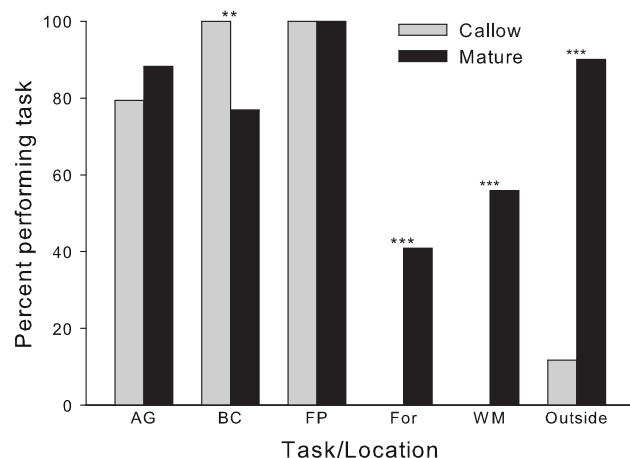
n number of observed workers

Fig. 1 Effects of worker age on task performance and spatial tendency in *P. californicus*. *Gray bars* represent callow workers; *black bars* represent mature workers. *AG* allogrooming; *BC* brood care; *FP* food processing; *For* foraging; *WM* waste management; *Outside* outside of nest. Values are percentages of workers in each age class that performed each task or exited the nest at least once, under either colony size treatment. Workers were pooled across colonies ($n_{callow} = 34$, $n_{mature} = 281$). False discovery rate B–Y adjusted α = 0.02 (6 Chi-squared tests; **$P \leq 0.01$, ***$P \leq 0.001$)

separately. Callow workers constituted just 5–16 % of colonies; when DOL$_{indiv-tasks}$ 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 seven between-individual behavioral correlations: interindividual consistency in food processing became significant in one colony and non-significant in another, while the rank orders of waste management and total task performance were no longer maintained in two and three colonies, respectively.

Effects of genotype

Mature workers from the two matrilines differed in their overall patterns of task performance in every case except for three small colonies (Table 3; Fig. 2). Task-specific G tests revealed significant matrilineal differences in brood care (6 out of 8 colonies), food processing (5 colonies), foraging (1 colony), and waste management (7 colonies). In four colonies (A, E, H, I), workers from one matriline were more likely to perform brood care, while the other matriline was more likely to perform waste management; colonies D and J exhibited non-significant trends in the same direction. We did not detect any other consistent patterns of covariance across tasks; because greater than 50 % of task performances were food processing in most colonies, inverse relationships between food processing and other tasks may simply reflect time tradeoffs. Total task performance did not differ between matrilines within colonies (nested ANOVA: $F_{8,197} = 1.34$, $P = 0.22$), although it did vary among colonies ($F_{7,197} = 2.18$, $P = 0.04$). Matriline effects could not be explained by differences in body size; head width varied among colonies (nested ANOVA: $F_{7,195} = 3.85$, $P = 0.001$), but not between matrilines within colonies ($F_{8,195} = 1.47$, $P = 0.17$).

Discussion

Colony size and division of labor

Holbrook et al. (2011) demonstrated that division of labor increases with colony size during early colony ontogeny and among unmanipulated, same-aged colonies of *P. californicus*.

Table 3 Differences between matrilines in overall distribution of task performance

Colony	n_1	n_2	Colony size	No. tasks	G_4	P
A	19	7	Small	488	43.50	<0.001*
			Large	552	39.70	<0.001*
			Pooled	1,040	59.24	<0.001*
B	14	16	Small	441	7.59	0.11
			Large	521	25.37	<0.001*
			Pooled	962	30.80	<0.001*
D	4	17	Small	352	7.98	0.093
			Large	353	11.51	0.021*
			Pooled	705	19.07	0.001*
E	17	16	Small	405	29.69	<0.001*
			Large	578	10.97	0.027*
			Pooled	983	34.95	<0.001*
F	9	17	Small	452	31.48	<0.001*
			Large	567	24.09	<0.001*
			Pooled	1,019	48.86	<0.001*
H	16	15	Small	598	12.64	0.013*
			Large	533	27.78	<0.001*
			Pooled	1,131	23.85	<0.001*
I	16	8	Small	441	27.74	<0.001*
			Large	434	11.85	0.019*
			Pooled	875	27.74	<0.001*
J	10	12	Small	368	10.35	0.035
			Large	438	23.09	<0.001*
			Pooled	806	31.48	<0.001*

G tests are from contingency tables of 2 matrilines versus 5 tasks. False discovery rate B–Y adjusted $\alpha = 0.027$ (3 tests per colony; * denotes significance)

n number of mature workers per matriline

Here, however, experimental variation in colony size over a short time period failed to elicit a consistent change in division of labor. Together, these findings suggest that the scaling of division of labor in *P. californicus* is not an emergent epiphenomenon produced by transitory behavioral responses (Jeanson et al., 2007), but instead results from longer-term effects of colony size on individual and collective task performance.

How might task specialization and division of labor be mechanistically integrated with colony size? Extended response threshold models propose that workers' task propensities are self-reinforced, or modified by task-related experience; 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). Model simulations incorporating self-reinforcement predict that colony growth can indirectly promote task specialization over time (Gautrais et al., 2002; Merkle and Middendorf,

2004). Alternatively, if the scaling of division of labor is adaptive (Bonner, 2004; Holbrook et al., 2011), it may be functionally regulated by control mechanisms operating at the level of the colony. For example, the distribution of response thresholds across workers and tasks could be developmentally modulated by colony size, in a process analogous to the social regulation of physical and temporal worker subcastes in other eusocial systems (Wheeler and Nijhout, 1984; Wilson, 1985; Beshers et al., 1999; Huang and Robinson, 1999). In either scenario, size-related changes in task specialization are likely mediated by physiological changes that delay individual responses and might even require worker turnover to substantially reorganize the colony's labor force (Robinson, 1992; Huang and Robinson, 1999; Robinson, 2009).

The scaling of division of labor may also be associated with covariates that were disrupted by our controlled experiment, which attempted to isolate the effect of colony size. Attributes such as age and caste structure, task demand, and interaction networks may change and interact with colony size to influence individual task decisions (Pacala et al., 1996; Anderson and McShea, 2001; Dornhaus et al., 2012). Few other studies have manipulated social insect colony size to test for causal effects on social organization (reviewed by Dornhaus et al., 2012), and to our knowledge none has quantified division of labor per se (a colony-level property whereby different individuals perform different tasks; Michener, 1974). When Wilson (1983) reduced colonies of *Atta cephalotes* leafcutter ants from about 10,000 to 236 workers, the body size distribution of newly produced workers reverted to that of an incipient colony; worker size was loosely associated with task performance. However, in another polymorphic ant, *Pheidole morrisi*, the behavioral repertoire of major workers was not affected by short-term changes in colony size alone (Brown and Traniello, 1998). Individual and colony responses may also depend on the degree of colony size variation; for example, honey bee (*Apis mellifera*) workers began foraging at younger ages following major reductions in colony size (Winston and Fergusson, 1985), but their temporal caste schedules did not shift in response to moderate size decreases (Kolmes and Winston, 1988). As in Holbrook et al. (2011), we tested a relatively small range of *P. californicus* colony sizes, which can reach several thousand workers in nature (Johnson, 2000).

Size effects on colony-level task allocation

In addition to dividing labor among workers and tasks, social insect colonies face the challenge of allocating their workforces to satisfy changing colony needs (Calabi and Traniello, 1989; Seeley, 1995; Gordon, 1996). We found variation in colony size to cause a shift in the overall pattern

Fig. 2 Distribution of task performance by *P. californicus* workers from different matrilines. One matriline per colony is shown in *gray*; the other is in *black*. *AG* allogrooming; *BC* brood care; *FP* food processing; *For* foraging; *WM* waste management. Values are percentages of total tasks performed by each matriline across colony size treatments. False discovery rate B–Y adjusted $\alpha = 0.022$ (5 *G* tests per colony; $*P \leq 0.022$, $**P \leq 0.01$, $***P \leq 0.001$)
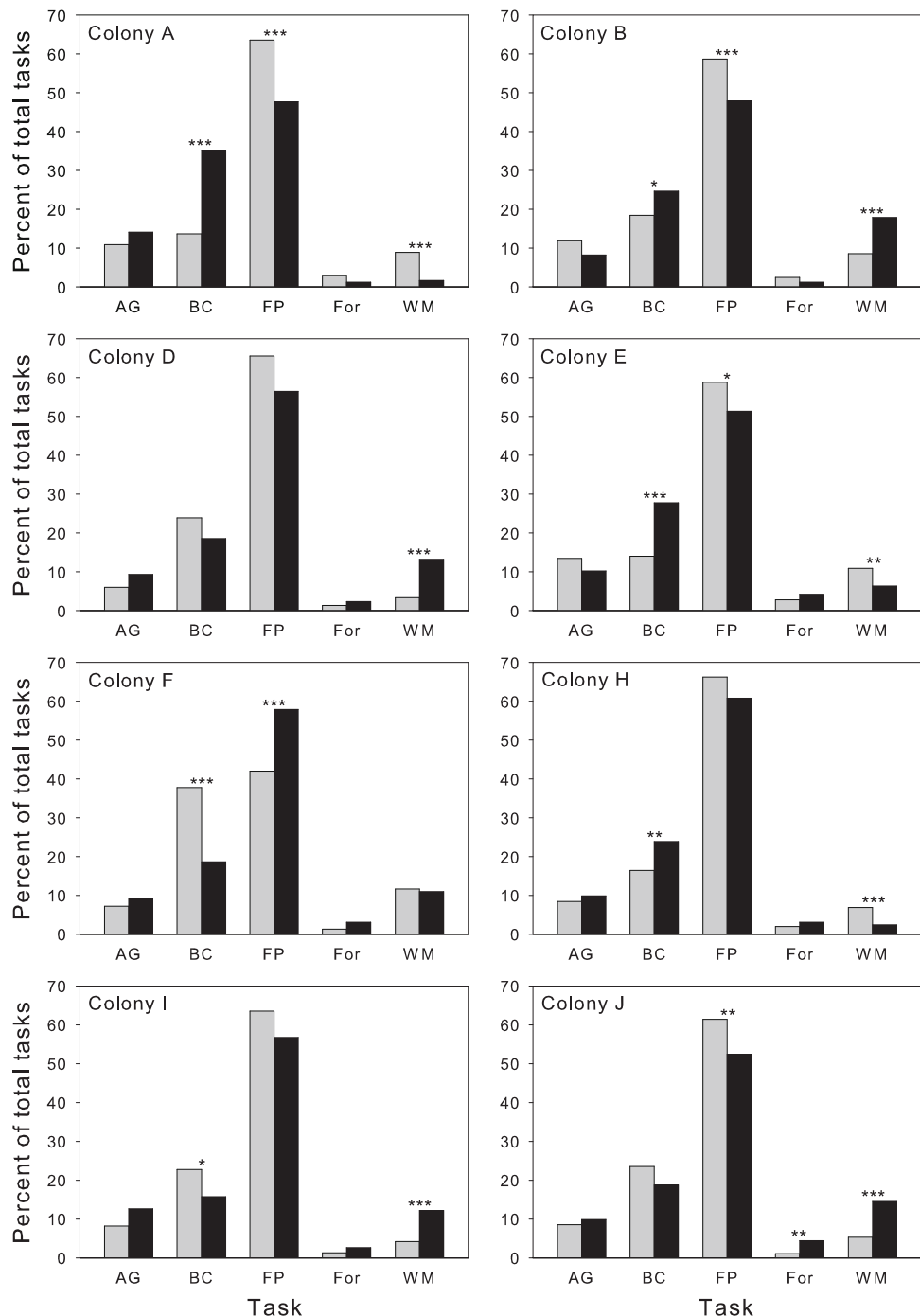
of task allocation. On a per capita basis, large colonies performed less brood care, more food processing, and more waste management than small colonies. In contrast, colony size did not affect the proportional allocation of workers to allogrooming, foraging, or total outside-nest activity. Changes in 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 that 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. Across all tasks, workers were more active (i.e., performed tasks more often) in larger colonies. These results are only partially consistent with those from unmanipulated *P. cali-*

fornicus colonies under similar laboratory conditions (Holbrook et al., 2011). In that study, workers also performed more brood care in younger, smaller colonies, and there was a weak trend toward increased waste management in older, larger colonies; however, total task performance per worker did not change with colony age, and there was no relationship between colony size and task allocation among same-aged colonies that varied naturally in size. This discrepancy may be explained, in part, by confounding sources of variation between unmanipulated colonies, such as demographic or genetic differences.

It is unclear why changes in colony size alone affected the proportional allocation of workers across tasks. According to the response threshold model, shifts in task allocation are driven by individual responses to local, task-specific stimuli (Beshers and Fewell, 2001). But we controlled the social and physical environment, other than colony size. The brood-to-worker ratio and nestmate density were held constant, and dispersion of brood within the nests of large colonies reduced potential crowding among brood care workers. Moreover, food was unlimited and individual foraging activity was independent of colony size. Therefore, the observed differences in brood care and food processing cannot easily be attributed to simple stimulus–response relationships. Waste management may have been stimulated by a higher rate of food processing in large colonies, if the latter generated more refuse per worker. An alternative explanation is that workers in large colonies did not actually process more food, but held onto their food items for longer periods of time before unloading them to larvae.

In tightly integrated, eusocial colonies, however, workers' task decisions are not based solely on independent responses to the environment, but can be coordinated through communication and social feedback (Hölldobler and Wilson, 1990, 2009; Seeley, 1995; Gordon, 1996). Thus, task needs or priorities may be calibrated by information on colony state (Schmid-Hempel et al., 1993; Tschinkel, 2006). Perhaps younger and/or smaller colonies are selected to invest 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., 2011).

Interindividual behavioral variability: intrinsic effects

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 may be caused by a number of factors, including worker age and genotype (Robinson, 1992; Beshers and Fewell, 2001).

Eusocial insects often exhibit age-related 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 (Hölldobler and Wilson, 1990; Traniello and Rosengaus, 1997). Individual behavioral development is associated with physiological changes, which may modulate workers' response thresholds for various tasks (Robinson, 1992; Robinson, 2009; Dolezal et al., 2012). 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 observed in the ant *Pheidole dentata* (Seid and Traniello, 2006); older *P. californicus* workers had larger behavioral repertoires that included inside- and outside-nest tasks. It should be noted, however, that temporal polyethism is quite variable in ants and can also depend on extrinsic factors such as nest architecture and colony needs (Traniello and Rosengaus, 1997; Gordon et al., 2005).

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., 2008a). Both sources of variation are present in populations of *P. californicus* (Overson, 2011). Our laboratory colonies contained two queens each, and in eight out of ten colonies, we identified multiple workers per matriline. Within those colonies, mature workers from the two matrilines differed in their patterns of task performance, but not in total activity. There was a tendency (significant in four colonies) for one matriline to perform a greater amount of brood care while the other performed relatively more waste management. Negative covariance between inside- and outside-nest tasks has been found in other polygynous ants as well (Snyder, 1992; Carlin et al., 1993; Julian and Fewell, 2004). Like Snyder (1992), our experimental design controlled for variation in rearing and posteclosion environment, suggesting that the observed matrilineal differences reflect genetic variation. However, other maternal effects cannot be ruled out, nor can the possible confounding influence of worker age, if queens lay eggs in asynchronous

bouts. Genotype is also known to influence workers' temporal polyethism schedules, which we did not quantify (Robinson, 1992; Julian and Fewell, 2004). Moreover, sample sizes were insufficient to analyze callow workers or to test for effects of patrilineal diversity within matrilines (Hughes et al., 2003; Jones et al., 2004; Smith et al., 2008b); therefore, the importance of intracolonial genetic variability for task organization in *P. californicus* may be underestimated.

Worker age, genotype, and other intrinsic properties are known to influence individual task decisions, yet we understand relatively little about how these factors interact with colony state parameters, including colony size, to organize the collective labor of eusocial colonies (Wilson, 1985; Robinson, 1992; Schmid-Hempel et al., 1993). Thus, Wilson's (1971, p. 227) "central problem" of insect sociobiology persists: explaining how the behaviors of individual colony members are integrated into a functional whole.

References

Anderson C. and McShea D.W. 2001. Individual versus social complexity, with particular reference to ant colonies. *Biol. Rev.* **76**: 211-237

Benjamini Y. and Yekutieli D. 2001. The control of the false discovery rate in multiple testing under dependency. *Ann. Stat.* **29**: 1165-1188

Beshers S.N. and Fewell J.H. 2001. Models of division of labor in social insects. *Annu. Rev. Entomol.* **46**: 413-440

Beshers S.N., Robinson G.E. and Mittenthal J.E. 1999. Response thresholds and division of labor in insect colonies. In: *Information Processing in Social Insects* (Detrain C., Deneubourg J.L. and Pasteels J.M., Eds), Birkhäuser Verlag, Basel, Switzerland. pp 115-139

Bonabeau E., Theraulaz G. and Deneubourg J.L. 1996. Quantitative study of the fixed threshold model for the regulation of division of labour in insect societies. *Proc. R. Soc. Lond. B.* **263**: 1565-1569

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

Brown J.J. and Traniello J.F.A. 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. *J. Insect Behav.* **11**: 209-219

Calabi P. and Traniello J.F.A. 1989. Behavioral flexibility in age castes of the ant *Pheidole dentata*. *J. Insect Behav.* **2**: 663-677

Carlin N.F., Reeve H.K. and Cover S.P. 1993. Kin discrimination and division of labour among matrilines in the polygynous carpenter ant, *Camponotus planatus*. In: *Queen Number and Sociality in Insects* (Keller L., Ed), Oxford University Press, Oxford, UK. pp 362-401

Chittka L. and Muller H. 2009. Learning, specialization, efficiency and task allocation in social insects. *Commun. Integr. Biol.* **2**: 151-154

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

Detrain C. and Pasteels J.M. 1992. Caste polyethism and collective defense in the ant, *Pheidole pallidula*: the outcome of quantitative differences in recruitment. *Behav. Ecol. Sociobiol.* **29**: 405-412

Dolezal A.G., Brent C.S., Hölldobler B. and Amdam G.V. 2012. Worker division of labor and endocrine physiology are associated in the harvester ant, *Pogonomyrmex californicus*. *J. Exper. Biol.* **215**: 454-460

Dornhaus A. 2008. Specialization does not predict individual efficiency in an ant. *PLoS Biol.* **6**: e285

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

Dornhaus A., Powell S. and Bengston S. 2012. Group size and its effects on collective organization. *Annu. Rev. Entomol.* **57**: 123-141

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

Fewell J.H. and Winston M.L. 1992. Colony state and regulation of pollen foraging in the honey bee, *Apis mellifera* L. *Behav. Ecol. Sociobiol.* **30**: 387-393

Gautrais J., Theraulaz G., Deneubourg J.L. and Anderson C. 2002. Emergent polyethism as a consequence of increased colony size in insect societies. *J. Theor. Biol.* **215**: 363-373

Gordon D.M. 1989. Dynamics of task switching in harvester ants. *Anim. Behav.* **38**: 194-204

Gordon D.M. 1996. The organization of work in social insect colonies. *Nature* **380**: 121-124

Gordon D.M., Chu J., Lillie A., Tissot M. and Pinter N. 2005. Variation in the transition from inside to outside work in the red harvester ant *Pogonomyrmex barbatus*. *Insect. Soc.* **52**: 212-217

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

Holbrook C.T. 2011. The emergence and scaling of division of labor in insect societies. Dissertation, Arizona State University

Holbrook C.T., Barden P.M. and Fewell J.H. 2011. Division of labor increases with colony size in the harvester ant *Pogonomyrmex californicus*. *Behav. Ecol.* **22**: 90-96

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

Hölldobler B. and Wilson E.O. 2009. *The Superorganism: The Beauty, Elegance, and Strangeness of Insect Societies*. W.W. Norton & Company, New York, NY

Holway D.A. and Case T.J. 2001. Effects of colony-level variation on competitive ability in the invasive Argentine ant. *Anim. Behav.* **61**: 1181-1192

Huang Z.Y. and Robinson G.E. 1999. Social control of division of labor in honey bee colonies. In: *Information Processing in Social Insects* (Detrain C., Deneubourg J.L. and Pasteels J.M., Eds), Birkhäuser, Basel, Switzerland. pp 165-186

Hughes W.O.H., Sumner S., Van Borm S. and Boomsma J.J. 2003. Worker caste polymorphism has a genetic basis in *Acromyrmex* leaf-cutting ants. *Proc. Natl Acad. Sci. USA* **100**: 9394-9397

Jeanne R.L. 1986. The organization of work in *Polybia occidentalis*: costs and benefits of specialization in a social wasp. *Behav. Ecol. Sociobiol.* **19**: 333-341

Jeanson R. and Fewell J.H. 2008. Influence of the social context on division of labor in ant foundress associations. *Behav. Ecol.* **19**: 567-574

Jeanson R., Fewell J.H., Gorelick R. and Bertram S.M. 2007. Emergence of increased division of labor as a function of group size. *Behav. Ecol. Sociobiol.* **62**: 289-298

Johnson R.A. 2000. Seed-harvester ants (Hymenoptera: Formicidae) of North America: an overview of ecology and biogeography. *Sociobiology* **36**: 89-122 + 183-188

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

Julian G.E. and Fewell J.H. 2004. Genetic variation and task specialization in the desert leaf-cutter ant, *Acromyrmex versicolor*. *Anim. Behav.* **68**: 1-8

Kaspari M. and Vargo E.L. 1995. Colony size as a buffer against seasonality: Bergmann's rule in social insects. *Am. Nat.* **145**: 610

Kolmes S.A. and Winston M.L. 1988. Division of labour among worker honey bees in demographically manipulated colonies. *Insect. Soc.* **35**: 262-270

Merkle D. and Middendorf M. 2004. Dynamic polyethism and competition for tasks in threshold reinforcement models of social insects. *Adapt. Behav.* **12**: 251

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

Nakagawa S. 2004. A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behav. Ecol.* **15**: 1044-1045

Narum S.R. 2006. Beyond Bonferroni: less conservative analyses for conservation genetics. *Conserv. Genet.* **7**: 783-787

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

Oldroyd B.P. and Fewell J.H. 2007. Genetic diversity promotes homeostasis in insect colonies. *Trends Ecol. Evol.* **22**: 408-413

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

Overson R.P. 2011. Causes and consequences of multi-queen colonies in the California harvester ant *Pogonomyrmex californicus*. Dissertation, Arizona State University

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

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

Pol R.G., de Casenave J.L., Feldhaar H., Milesi F.A. and Gadau J. 2008. Polyandry in two South American harvester ants. *Insect. Soc.* **55**: 91-97

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

Robinson E. 2009. Physiology as a caste-defining feature. *Insect. Soc.* **56**: 1-6

Robinson G.E. 1992. Regulation of division of labor in insect societies. *Annu. Rev. Entomol.* **37**: 637-665

Robinson G.E. and Page R.E. 1989. Genetic basis for division of labor in an insect society. In: *The Genetics of Social Evolution* (Breed M.D. and Page R.E., Eds), Westview, Boulder, CO. pp 61-80

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

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

Seid M.A. and Traniello J.F.A. 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. *Behav. Ecol. Sociobiol.* **60**: 631-644

Smith C., Toth A., Suarez A. and Robinson G. 2008a. Genetic and genomic analyses of the division of labour in insect societies. *Nat. Rev. Genet.* **9**: 735-748

Smith C.R., Anderson K.E., Tillberg C.V., Gadau J. and Suarez A.V. 2008b. Caste determination in a polymorphic social insect: nutritional, social, and genetic factors. *Am. Nat.* **172**: 497-507

Snyder L.E. 1992. The genetics of social behavior in a polygynous ant. *Naturwissenschaften* **79**: 525-527

Theraulaz G., Bonabeau E. and Deneubourg J.L. 1998. Response threshold reinforcement and division of labour in insect societies. *Proc. R. Soc. Lond. B.* **265**: 327-332

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

Traniello J.F.A. and Rosengaus R.B. 1997. Ecology, evolution and division of labour in social insects. *Anim. Behav.* **53**: 209-213

Tschinkel W.R. 2006. *The Fire Ants.* The Belknap Press of Harvard University Press, Cambridge, MA

Volny V.P. and Gordon D.M. 2002. Characterization of polymorphic microsatellite loci in the red harvester ant, *Pogonomyrmex barbatus*. *Mol. Ecol. Notes* **2**: 302-303

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

Wheeler D.E. and Nijhout H.F. 1984. Soldier determination in *Pheidole bicarinata*: inhibition by adult soldiers. *J. Insect Physiol.* **30**: 127-135

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

Wilson E.O. 1983. Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*). IV. Colony ontogeny of *A. cephalotes*. *Behav. Ecol. Sociobiol.* **14**: 55-60

Wilson E.O. 1985. The sociogenesis of insect colonies. *Science* **228**: 1489-1495

Winston M.L. and Fergusson L.A. 1985. The effect of worker loss on temporal caste structure in colonies of the honeybee (*Apis mellifera* L.). *Can. J. Zool.* **63**: 777-780