

# Ant stoichiometry: elemental homeostasis in stage-structured colonies

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## Summary

1. Organisms facing variation in food quality maintain elemental composition within limited bounds. Such stoichiometric homeostasis has often been considered a species-specific parameter, but stoichiometry can also vary intraspecifically across life stages, sexes and sizes. In colonial organisms with overlapping generations, stoichiometric variation among stages could lead to flexibility in colony-level elemental composition due to changes in internal demography.
2. We examine how the balance of energy (sucrose) and nutrients (prey) affects growth rate and carbon : nitrogen : phosphorus (C : N : P) homeostasis in a eusocial insect, the pavement ant *Tetramorium caespitum*.
3. Colony growth depended heavily on prey availability. However, sucrose scarcity led to higher worker mortality and production of smaller workers, suggesting sucrose availability will affect colony-level performance in a competitive environment.
4. In contrast, C : N : P stoichiometry of larvae, pupae, and workers varied mostly with sucrose availability. Biomass P content within life stages was lower in colonies receiving less access to sucrose. We suggest this difference arose primarily from shifts in individual ant mass coupled with negative P-body mass relationships.
5. Life stages differed considerably in elemental composition, and resource conditions affected colony stage structure. Nevertheless, variation in colony-level stoichiometry primarily reflected compositional differences within stages rather than shifts in internal demography.

*Key-words:* carbohydrates, growth rate hypothesis, nutrient balance, phosphorus, RNA

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## Introduction

Homeostasis has served as a conceptual framework for animal physiologists for over a century (Bernard 1872; Randall, Burggren & French 2001). More recently, it has emerged as a central idea in ecological stoichiometry, the study of the balance of energy and materials in living systems (Sternler & Elser 2002). The degree to which organisms regulate elemental composition may influence the magnitude and nature of their ecological impact. For example, strict elemental homeostasis may result in consumers disproportionately depleting resources containing scarce materials and increasing recycling rates of materials ingested in excess of demand (Vanni 2002). Alternatively, elemental composition may be flexible, and stoichiometric shifts in individuals

could reflect changes in functional capabilities (Kay *et al.* 2005). For example, the growth rate hypothesis (GRH) predicts a causal link between variation in whole-body P concentration and growth rate, a key life-history attribute, due to the role that P-rich ribosomal RNA plays in regulating protein synthesis (Elser *et al.* 1996). Elucidating the degree of homeostasis in different taxa and the factors that explain regulatory variation should help clarify the mechanisms that link food quality to consumer traits that affect ecological interactions (Sternler & Elser 2002).

In this paper, we examine elemental homeostasis at the individual- and colony-level in the pavement ant, *Tetramorium caespitum*. No previous study has examined the C : N : P stoichiometry of a social insect, but this group may prove to be an important system for exploring the maintenance and ecological relevance of elemental homeostasis. Relative to solitary animals, ants and other social insects may be more likely to vary in chemical composition in response to changing food quality due to within-colony specializations. Social insect colonies contain multiple life stages with

different nutritional requirements, which are reflected in the pattern of food flow through colonies: workers shunt nutrients to queens and larvae but retain carbohydrates (Sorenson & Vinson 1981; Weeks *et al.* 2004). It is not yet known whether stoichiometric differences among life stages reflect nutritional allocation within a colony. However, elemental composition can vary with ontogeny in aquatic invertebrates, with juvenile stages generally having higher P concentrations than adults (Villar-Argaiz, Medina-Sánchez & Carillo 2002; DeMott 2003). This difference presumably reflects higher RNA requirements for protein synthesis during development. If P concentrations are significantly higher in ant larvae than in adult workers, C : P stoichiometry at the colony-level should shift with the relative rates of worker production and mortality. Colonies composed primarily of developing larvae may thus have both higher specific growth rate and higher P concentration, which would provide support for the GRH in a social rather than purely individual context. Many ant species collect multiple chemically distinct resources (Way 1963; Hölldobler & Wilson 1990), such as nectars with high C : nutrient ratios and invertebrates richer in N and P. As a result, colonies may often face variation in elemental supply ratios (Kay 2002). Flexible colony-level stoichiometry could complement pre-digestive mechanisms for nutrient balancing (Raubenheimer & Simpson 1997), such as selective foraging behaviour (Kay 2004; Mayntz *et al.* 2005), to increase production in the face of such variation.

Here, we explore how the availabilities of an energy-rich resource (sucrose) and a nutrient-rich resource (prey) affect colony growth, internal demography, and the C : N : P stoichiometry and RNA content of individuals and colonies. Specifically, we ask whether sucrose availability and prey availability differentially affect worker survival and new worker production, leading to diet-related changes in the brood : worker ratio in colonies. We also test whether life stages differ in C : N : P stoichiometry, and whether a change in the availability of a nutritionally distinct resource induces a shift in individual ant stoichiometry and a change in whole-colony stoichiometry due to demographic alterations. Alternatively, there could be strict elemental homeostasis in individual ants and whole colonies across resource conditions. Finally, we test whether diet-induced variation in whole-colony P concentration is correlated with specific growth rate, consistent with the GRH.

Determining the effect of resource quality on colony growth and demography should provide information on bottom-up controls of ecological interactions among ants because colony size and life stage structure have important effects on exploitative foraging ability (Holway & Case 2001) and territorial prowess (Adams 2003). Stoichiometry could also influence the performance of individuals, but virtually nothing is known about variation in ant elemental composition, much

less the functional consequences of any variation (but see Davidson 2005). Investigating the causes of stoichiometric variation in a social organism extends the purview of ecological stoichiometry and should create new opportunities for determining how nutrient constraints affect the economics of social interactions.

## Methods

Newly mated *T. caespitum* foundresses were collected in 2002. Colonies of this introduced species scavenge for invertebrates and collect root exudates and other nectars (Leimar & Axen 1993). Colonies were reared on honey-egg agar and mealworms.

For the experiment, 18 single-queen colonies were each assigned to one of six groups (three colonies per group). Initially, larval mass, pupal number, and worker number were standardized across colonies within groups, and queen mass and the collective masses of workers and pupae for each colony were measured (Table 1). Colonies were housed in glass tubes half-filled with water trapped in with cotton and placed in plastic containers lined with fluon and sticky trap (Tanglefoot). Colonies were kept on 16 h : 8 h light : dark and 28 °C : 20 °C temperature schedules.

Experimental colonies were provided with sucrose agar and 'prey', which consisted of fruit flies *Drosophila melanogaster* and tuna. Colonies within groups were haphazardly assigned one of three treatments in which colonies were given access to: a high-sucrose food agar and low amounts of prey (hereafter, HSLP), high-sucrose agar and high prey (HSHP), or low-sucrose agar and high prey (LSHP), as described below. To standardize agar and prey availability within treatments across groups, food quantity was scaled by the initial number of workers in a colony. Colonies were fed 5 out of every 7 days; colonies never went more than 1 day without food. Food remains were removed once per week. The amount of food given the HSHP treatment provided colonies with the opportunity for approximately *ad libitum* consumption; HSLP and LSHP treatments were designed to test the effects of prey and sucrose scarcity, respectively. Per gram of food, the sucrose agar contained a completely homogenized mixture of 30 mg egg, 0.2 mg Na<sub>2</sub>CO<sub>3</sub>, 0.5 mg

**Table 1.** Initial masses of larvae, pupae, and workers (mean  $\pm$  1 SE, among colonies within group) for each group of experimental *Tetramorium caespitum* colonies

Colony group	Larval mass (mg)	Pupal mass (mg)	Worker mass (mg)
1	39.4 $\pm$ 0.5	49.4 $\pm$ 0.7	278.5 $\pm$ 0.7
2	110.8 $\pm$ 1.6	3.0 $\pm$ 0.3	192.4 $\pm$ 9.9
3	71.9 $\pm$ 2.2	20.6 $\pm$ 1.0	143.7 $\pm$ 2.0
4	62.1 $\pm$ 2.2	53.1 $\pm$ 2.9	106.0 $\pm$ 7.2
5	28.7 $\pm$ 2.0	30.5 $\pm$ 1.2	98.2 $\pm$ 4.4
6	6.3 $\pm$ 0.2	4.3 $\pm$ 0.5	20.8 $\pm$ 0.7

$K_2HPO_4$ , 0.15 mg  $MgSO_4$ , 0.15 mg CaCl, 20 ng thiamine HCl, 10 pg of both biotin and vitamin B12, and either 20 mg or 200 mg sucrose in the low- and high-sucrose food, respectively; egg was measured by wet mass, all other substances were measured by dry mass. Colonies in all three treatments received the same amount of agar, and thus had equal access to egg, minerals and vitamins. Both fruit flies and tuna were used as prey because preliminary results suggested these resources had synergistic effects on colony growth. We did not include a low sucrose, low prey treatment because preliminary results suggested queen mortality would be high in this treatment.

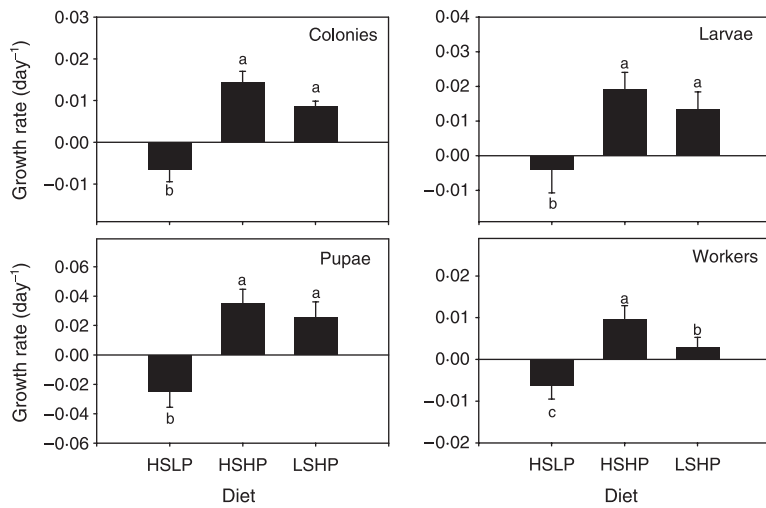
At the end of each week, dead workers in each container were counted and removed. After 56 days, queen mass, the collective masses of larvae, pupae and workers, and the number of pupae and workers in each colony were measured. In addition, individual dry masses of 10–15 pupae, young workers, and older workers in each colony were haphazardly selected and measured separately to assess how diet treatment affected the size of individuals produced during the experiment. Young (newly eclosed) workers are clearly distinguishable from older workers by their lighter colour. Specific growth rate over the 56-day period was estimated using the formula: growth rate ( $day^{-1}$ ) =  $\ln(\text{final wet mass}/\text{initial wet mass})/56$ .

At the end of the experiment, larvae, pupae and workers were haphazardly collected for chemical analyses. Before harvesting, colonies were starved for 24 h to reduce the effect of recent consumption on ant elemental composition. To estimate colony-level stoichiometry, large composite samples containing 0.5–6 mg of larval, pupal or worker dry mass were created from each colony in five groups. Each composite sample was divided into four subsamples (two for P analysis, two for C and N analysis). A single measure for each colony (total elemental content/total dry mass of samples) was calculated for each life stage for use in statistical comparisons of composite samples. In addition, we sampled larvae, pupae and workers of various sizes to assess the effect of diet and body size on individual ant stoichiometry. For P, 10 larvae, five pupae and 10 workers from each colony were analysed. For C and N, five larvae, three pupae and three workers from these same colonies were analysed, although colonies in one group were too small to yield sufficient larval samples for comparison. In addition, RNA in eight larvae and five workers were measured for three groups containing large colonies. To assess the stoichiometry of available resources, three samples of eggs, 10 flies, and tuna were analysed for C, N and P. The C : N : P stoichiometry of the digestible portion of the sucrose food was estimated using our analysis of egg composition and the chemical formula of sucrose. The C : N : P stoichiometry of available resources was 304 : 11 : 1, 84 : 9 : 1 and 47 : 9 : 1 for the HSLP, HSHP and LSHP treatments, respectively. Throughout, all elemental ratios are molar.

For P, C and N concentrations, analysed samples were first dried at 50 °C for 48 h. P concentrations were measured by persulfate digestion followed by colorimetric analysis (as described by Woods *et al.* 2002) on an ALPKEM Flow Solution 3000 analyser. Prior to digestion, individuals were gently crushed with a Teflon-coated rod while in solution to expose internal tissue to reagents. Composite samples were crushed with a mortar and pestle while dry. A separate set of samples were analysed for C and N using a Perkin-Elmer 2400 CHN analyser. In rare cases when larvae were too small for individual CHN analysis, individuals of similar mass were combined in a sample and average individual mass was used in the statistical analysis. Concentrations of C, N and P in a colony were estimated from the elemental content in all analysed samples for each life stage, weighted by the relative dry masses of life stages in colonies at the end of the growth experiment. Total dry masses for larvae, pupae and workers in colonies were estimated from wet masses using conversions (0.296, 0.232, 0.344) based on dry mass : wet mass comparisons for 25 individuals per life stage. Queens were not included in calculations of whole-colony elemental concentrations; on average, queens comprised  $5.6 \pm 1.8\%$  (mean  $\pm$  1 SE) of colony mass.

The concentration of RNA was measured in samples maintained at  $-70$  °C until analysis. The RNA assay (as described in Kyle *et al.* 2003) involves extraction with N-laurylarcosine, sonication and staining with Ribogreen (Molecular Probes, Eugene, OR, USA). RNA content was compared with dry mass using the parameters of relationships between frozen mass and dry mass (determined separately for larvae and workers using 25 individuals in each life stage from each of two colonies).

All data except those for growth rate were  $\log_{10}$ -transformed before analysis. Growth rate and elemental concentrations in composite samples were compared among treatments using ANOVA (blocking for colony group), and worker mortality was compared using repeated-measures ANOVA. Throughout, Tukey's Honest Significant Difference tests were used for pairwise comparisons following significant ANOVAs. Concentrations of C, N, P and RNA in individuals were compared using GLM (General Linear Models) with diet treatment, colony (nested within diet), and life stage as independent variables; individual dry mass was included as a covariate. Colony was treated as a fixed effect. Nutrient concentrations were also analysed separately for each life stage. In all GLM analyses, interaction terms that were not significant were dropped from the final models. We also used a nested analysis to compare individual pupa and worker dry mass to diet treatment (with colony nested within diet). MANOVA was used to explore the effect of treatment on colony C : P, N : P, or C : N ratios and the ratio of immature (pupae and larvae) dry mass to adult worker dry mass in colonies. Immature and adult dry



**Fig. 1.** Mean + 1 SE daily specific growth rate vs. diet treatment for whole colonies and colony components (larvae, pupae, workers) over a 56-day period. Diet treatments are: HSLP = high sucrose, low prey; HSHP = high sucrose, high prey; LSHP = low sucrose, high prey. Different letters indicate significant differences ( $P < 0.05$ ) in pairwise comparisons.

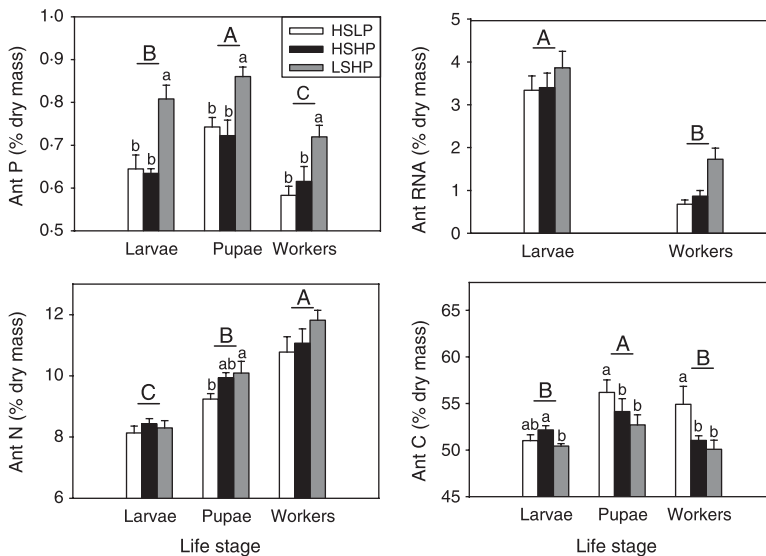
masses were estimated from wet masses using conversion factors described above. A significant MANOVA was followed by univariate ANOVAs. Colony growth rate was also compared with colony C : P, N : P and C : N ratios using linear regression.

## Results

Prey scarcity affected colony growth much more than sucrose scarcity did (Fig. 1). Diet treatment

significantly affected overall colony biomass gain ( $F_{2,10} = 26.82$ ,  $P < 0.001$ ), and mass change in larval ( $F_{2,10} = 16.68$ ,  $P = 0.001$ ) and pupal ( $F_{2,10} = 13.28$ ,  $P < 0.001$ ) populations. In each comparison, growth rate for colonies in the low prey (HSLP) treatment was significantly lower than for colonies in the high prey (HSHP or LSHP) treatments (Fig. 1). In contrast, change in the mass of worker populations was affected by both prey and sucrose scarcity, as it was significantly less in the HSLP and the LSHP treatments than in the HSHP treatment (Fig. 1; overall ANOVA:  $F_{2,10} = 20.88$ ,  $P < 0.001$ ). Worker mortality also differed significantly among treatments ( $F_{2,10} = 4.21$ ,  $P = 0.047$ ). Worker mortality in colonies in the low sucrose (LSHP) treatment (0.011 workers/no. initial worker per day) was 86% and 68% higher than those in the HSLP and HSHP treatments, respectively, although pairwise differences were not quite statistically significant (LSHP vs. HSLP,  $P = 0.056$ , LSHP vs. HSHP:  $P = 0.097$ ). At the end of the experiment, dry mass for individual young workers differed significantly among treatments ( $F_{2,15} = 6.911$ ,  $P = 0.007$ ) and was significantly lower in LSHP colonies than in HSLP colonies ( $P = 0.007$ ) or in HSHP colonies ( $P = 0.050$ ). Similarly, individual pupal dry mass differed significantly among treatments ( $F_{2,15} = 4.29$ ,  $P = 0.034$ ) and was lowest in LSHP colonies, although pairwise comparisons were not quite significant (LSHP vs. HSLP,  $P = 0.064$ , LSHP vs. HSHP:  $P = 0.086$ ). In contrast, individual dry mass for older workers did not differ among treatments ( $F_{2,15} = 0.04$ ,  $P = 0.961$ ). Changes in queen mass also did not differ among treatments ( $F_{2,10} = 0.98$ ,  $P = 0.474$ ); no queens died during the experiment.

In contrast to colony growth, P and RNA concentrations in colony members varied more with sucrose availability than prey availability (Fig. 2). In composite samples, diet treatment significantly affected P concentrations in larvae, pupae and older workers (Table 2). For all life stages, biomass in colonies receiving the low sucrose (LSHP) treatment had significantly higher P concentrations than that in colonies receiving high sucrose (HSLP and HSHP) treatments (Fig. 2); there were no significant differences in any HSLP vs. HSHP comparison, indicating that prey availability did not affect P concentrations in any life stage. With individual dry mass as a covariate, diet treatment significantly affected P concentration in workers ( $F_{2,15} = 5.60$ ,  $P = 0.015$ ; Fig. 3) but not in larvae ( $F_{2,15} = 2.49$ ,  $P = 0.121$ ) or pupae ( $F_{2,15} = 1.98$ ,  $P = 0.173$ ). For each life stage, most of the variation in P concentration among individual ants was explained by negative relationships with dry mass (larvae:  $F_{1,124} = 588.63$ ,  $P < 0.001$ ,  $\log[P] = -0.32 * \log \text{dry mass} - 0.38$ ; pupae:  $F_{1,57} = 47.84$ ,  $P < 0.001$ ,  $\log[P] = -0.65 * \log \text{dry mass} - 0.64$ ; workers:  $F_{1,70} = 90.25$ ,  $P < 0.001$ ,  $\log[P] = -0.62 * \log \text{dry mass} - 0.66$ ). RNA concentration in individual workers also differed significantly among treatments with individual dry mass as a covariate

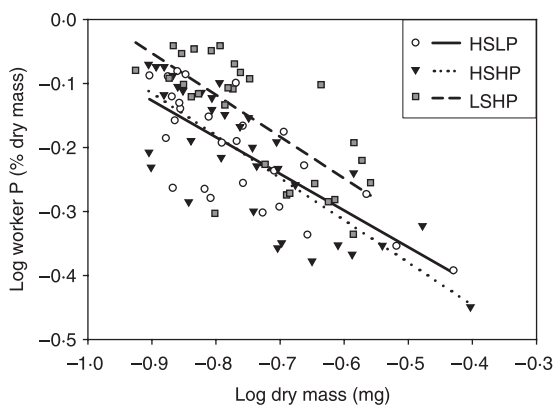


**Fig. 2.** Mean + 1 SE phosphorus (P), RNA, nitrogen (N) and carbon (C) concentrations (% dry mass) for larvae, pupae and workers from colonies given different access to sucrose and prey over a 56-day period. Data for elemental concentrations are from composite samples containing multiple individuals; data for RNA are from individuals ants. Diet treatments are: HSLP = high sucrose, low prey; HSHP = high sucrose, high prey; LSHP = low sucrose, high prey. Different upper case letters above bar trios indicate significant differences in pairwise comparisons between life stages. Different lower case letters within bar trios indicate significant differences between diets within life stages.

**Table 2.** Dependence of phosphorus (P), nitrogen (N) and carbon (C) concentrations (% dry mass) in biomass of *Tetramorium caespitum* life stages on sucrose and prey availability (= diet) and colony group. Dependent variables are total elemental content/total dry mass of all samples analysed for each colony

			P	N	C
Life Stage	Variable	d.f.	<i>F</i> -ratio	<i>F</i> -ratio	<i>F</i> -ratio
Larvae	Diet	2	21.24***	0.90	8.92**
	Colony group	5	2.84	3.46*	5.72**
	Error	10			
Pupae	Diet	2	15.89***	4.94*	19.65***
	Colony group	5	4.61*	2.96	29.46***
	Error	10			
Workers	Diet	2	15.61***	2.08	9.15**
	Colony group	5	5.29*	2.17	4.91*
	Error	10			

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



**Fig. 3.** Log-log relationship between whole-body P concentration and dry mass in ant workers from colonies fed different diets. Diet treatments are: HSLP = high sucrose, low prey; HSHP = high sucrose, high prey; LSHP = low sucrose, high prey.

( $F_{2,6} = 5.21$ ,  $P = 0.049$ ) and was higher in LSHP colonies than in HSLP or HSHP colonies (Fig. 2). Larval RNA concentration did not differ significantly among treatments ( $F_{2,6} = 0.37$ ,  $P = 0.703$ ; Fig. 2). In workers, allocation to RNA explained 69% and 75% of the individual mass-specific P variation in LSHP–HSHP and LSHP–HSHP comparisons of colony means. RNA concentration decreased significantly with larval dry mass ( $F_{1,61} = 17.47$ ,  $P < 0.001$ ; all samples:  $\log[\text{RNA}] = -0.155 * \log \text{dry mass} + 0.407$ ), but did not vary significantly with worker mass ( $F_{1,35} = 2.58$ ,  $P = 0.117$ ).

Diet treatment also significantly affected N and C concentrations in composite samples (Table 2, Fig. 2): pupal biomass from LSHP colonies contained significantly more N than pupae from LSHP colonies, and worker biomass from HSLP colonies contained higher C concentrations than that from high prey treatments (HSHP, LSHP). In contrast, diet treatment did not significantly affect N or C concentrations in individual ants with dry mass included as a covariate (N: larvae –  $F_{2,12} = 0.69$ ,  $P = 0.527$ ; pupae –  $F_{2,12} = 1.44$ ,  $P = 0.270$ ; workers –  $F_{2,12} = 3.65$ ,  $P = 0.058$ ; C: larvae –  $F_{2,12} =$

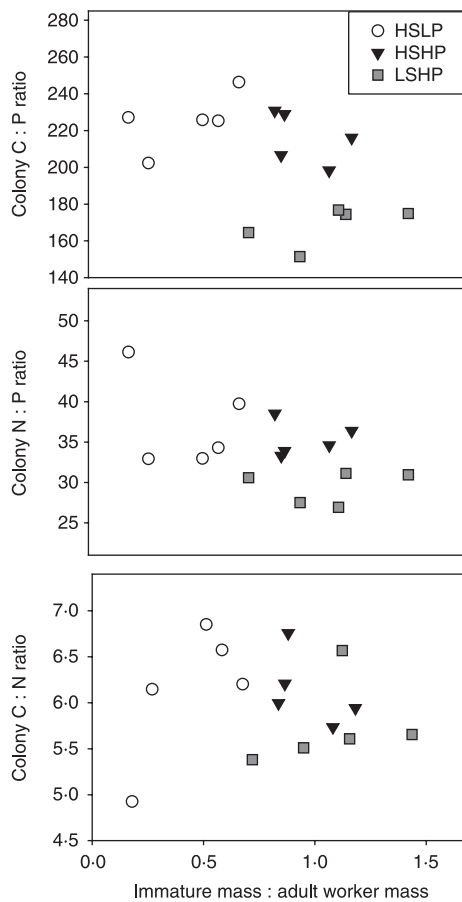
1.60,  $P = 0.240$ ; pupae –  $F_{2,12} = 0.40$ ,  $P = 0.680$ ; workers –  $F_{2,12} = 1.24$ ,  $P = 0.329$ ). C concentration significantly increased with mass in larvae ( $F_{1,56} = 5.52$ ,  $P = 0.02$ ,  $\log[\text{C}] = 0.037 * \log \text{dry mass} + 1.75$ ), and N concentration significantly decreased with mass in pupae ( $F_{1,27} = 21.21$ ,  $P < 0.001$ ,  $\log[\text{N}] = -0.37 * \log \text{dry mass} + 0.73$ ) and workers ( $F_{1,34} = 18.72$ ,  $P = 0.001$ ,  $\log[\text{N}] = -0.15 * \log \text{dry mass} + 0.98$ ).

Elemental concentrations also differed among life stages in composite samples (phosphorus:  $F_{2,44} = 30.84$ ,  $P < 0.001$ ; nitrogen:  $F_{2,44} = 96.17$ ,  $P < 0.001$ ; carbon:  $F_{2,44} = 12.42$ ,  $P < 0.001$ ; Fig. 2). P concentration was significantly higher in immature stages than in workers, N concentration was higher in later life stages, and C concentration was significantly lower in larvae and workers than in pupae. For RNA, concentrations in individual larvae were significantly higher than those in workers when ant dry mass was included as a covariate ( $F_{1,6} = 46.50$ ,  $P < 0.001$ ; Fig. 2).

Diet treatment affected the relationship between colony stoichiometry and stage structure (Fig. 4). Colonies in the low prey (HSLP) treatment contained significantly less immature mass (larvae and pupae) relative to worker mass than did HSHP or LSHP colonies, while colony-level C : P and N : P ratios were significantly lower for colonies in the low sucrose (LSHP) treatment than for those in the high sucrose treatments (Table 3). C : N ratios in colonies did not differ significantly with diet treatment (Table 3). Across all colonies, growth rate was significantly negatively correlated with colony C : P ( $r^2 = 0.261$ ,  $P = 0.012$ ) and N : P ( $r^2 = 0.314$ ,  $P = 0.007$ ) ratios, but did not vary significantly with colony C : N ratio ( $r^2 = 0.009$ ,  $P = 0.636$ ).

## Discussion

The availability of both sucrose and prey affected the growth rate of pavement ant colonies and the C : N : P stoichiometry of colony members within each major life stage. Elemental ratios also differed among life stages, and diet treatment induced a change



**Fig. 4.** Colony-level stoichiometric molar ratios vs. the ratio of the dry mass of immature workers (larvae and pupae) to adult worker dry mass for colonies fed different diets. C = carbon, P = phosphorus, N = nitrogen. Diet treatments are: HSLP = high sucrose, low prey; HSHP = high sucrose, high prey; LSHP = low sucrose, high prey.

in life stage structure within colonies. These findings suggest that independent variation in nectar and prey availability influences individual- and colony-level features that likely impact competitive interactions.

**Table 3.** Diet treatment effects on element ratios and stage structure. Stage structure is the ratio of the mass of immature workers (larvae and pupae) to adult worker mass. C = carbon, P = phosphorus, N = nitrogen. All stoichiometric ratios are molar. Results are from (a) MANOVAS and (b) univariate ANOVAS

(a) manovas			
Variables (d.f. = 4,14)	Wilks' lambda	Rao's <i>F</i>	<i>P</i>
Colony C : P ratio, immature mass : adult mass	0.01	27.06	< 0.001
Colony N : P ratio, immature mass : adult mass	0.09	7.99	0.001
Colony C : N ratio, immature mass : adult mass	0.11	7.32	0.002
(b) univariate ANOVAS			
Variable (d.f. = 2,8)	<i>F</i>		<i>P</i>
Immature mass : adult mass	10.30		0.006
Colony C : P ratio	127.48		< 0.001
Colony N : P ratio	10.56		0.006
Colony C : N ratio	1.31		0.321

Colony-level growth depended much more on the availability of prey than on sucrose availability (Fig. 1). Larval growth in the HSLP treatment was likely limited by one or more materials abundant in prey. Although protein limitation is an obvious candidate (Scriber & Slansky 1981), a lack of dietary P can also reduce growth rates in insects (Perkins *et al.* 2004). Other studies have shown that egg and larval production declines significantly if ant colonies are fed sucrose but denied nutrient-rich supplements (Evans & Pierce 1995). Indeed, such nutritional constraints are thought to be a key factor favouring the exploitation of higher trophic levels by omnivores (White 1993).

The effect of sucrose availability on colony growth was more subtle than the effect of prey. Colonies given the LSHP treatment produced as much larval and pupal mass as colonies given the HSHP treatment, but gained less worker mass (Fig. 1) presumably due in part to differences in worker mortality. Colonies given the low sucrose (LSHP) treatment also appear to have produced smaller workers during the experiment, as young workers but not older workers in these colonies were significantly smaller than those from colonies given access to high sucrose agars. We speculate that these effects reflect a shortage of carbohydrate fuel for worker maintenance and production. Regardless of the mechanism, these changes in worker number and size suggest that the impact of carbohydrate availability on production may not be fully realized outside of a competitive context. The number and sizes of workers are key determinants of exploitation and interference competition among ant colonies (Holway & Case 2001; Adams 2003). Interference ability in particular may be critical for pavement ants, which have protracted battles involving masses of workers engaged in physical combat (McCook 1880). Future work should assess the extent to which carbohydrate shortages limit colony performance in these battles and other competitive activities. In contrast to our results, Bono & Herbers (2003) found *Myrmica brevispinosa* colonies produced smaller workers after protein supplementation, whereas carbohydrate supplementation had no effect on worker mass. Diet quality may thus prove to be an important determinant of worker size, creating a linkage between the relative availabilities of nutrients and colony performance. However, the nature of this linkage may differ among species.

The major stoichiometric difference among individuals was the higher P concentration in larval, pupal and worker biomass in composite samples from LSHP colonies relative to that from HSLP and HSHP colonies; N concentration in composite samples differed only in pupae, while C concentration differed modestly in all three life stages (Fig. 2). The relatively large variation in P vs. C and N concentrations is consistent with stoichiometric patterns across zooplankton taxa (Sterner & Elser 2002, p. 139), and suggests that

a focus on P in social insects may reveal linkages among composition, biological functions and life-history strategies. These differences arose even though colonies were given sucrose and prey items independently and thus through selective foraging could have ingested the same ratio of sucrose : prey in all treatments.

It seems intuitive that if consumer P concentration varied with diet quality, it would increase with the availability of P in potential food sources. This result has been found in several aquatic and terrestrial invertebrates (DeMott 2003; Perkins *et al.* 2004). In contrast, we found P concentration in colony members was not affected by the availability of P-rich prey, but instead was higher when sucrose was less available. The basis of these differences is also surprising. Analyses of individual ants suggest within-stage differences in biomass P likely reflect the combined effects of negative P allometries, individual mass differences, and differences in mass-specific P concentrations. At least in pupae and workers, higher P concentrations in LSHP colonies may largely be accounted for by a strong negative relationship between P concentration and individual ant body mass in each life stage coupled with the smaller masses of pupae and young workers in LSHP colonies. Thus, we suggest diet differences led to changes in individual ant mass which in turn created stoichiometric differences among colonies because of negative P-body mass relationships. This explanation could also account for among-treatment differences in larval P (which also decreased with mass in analyses of individuals), but we do not have data on average larvae masses in colonies. Individual workers in LSHP colonies also had higher mass-specific P concentrations than those from high sucrose colonies (Fig. 3), and similar, though not significant, differences existed in individual larvae and pupae; these mass-specific differences likely also contributed to P content differences in composite samples (Fig. 2).

In several taxa, P concentration varies with RNA levels and development rate, consistent with the GRH (Elser *et al.* 2003). Similarly, ant larvae in our study had higher P and RNA concentrations than did workers, which do not grow. However, differences in mass-specific RNA-P across diet treatments explained much of the variation in worker P, which cannot be directly related to growth rate. We suggest two mechanisms that could generate diet-induced variation in mass-specific worker P and RNA levels. First, workers may have faced increased rates of protein catabolism for use as metabolic fuel when colonies faced sucrose scarcity, and high RNA concentrations may reflect an increased rate of synthesis required for protein replacement. This process would be analogous to the colony-level pattern in the LSHP treatment of high levels of worker mortality and larval production. Second, differences in P concentrations among workers could reflect changes in allocation to stores of P-free lipids and carbohydrates, or to exoskeleton, which generally contains less

P than most soft tissue in insects (Hackman 1984). Variable allocation to exoskeleton was one mechanism proposed by Woods *et al.* (2004) to explain inter-specific variation in P content among adult insects. Less allocation to energy storage or exoskeleton would increase whole body RNA concentration, even if RNA concentrations in metabolically active soft tissue did not change. If colonies facing sucrose shortages produce workers with less energy reserves or exoskeleton, it should lead to further consequences for colony performance in a competitive setting.

We predicted that colony-level stoichiometry would vary with diet-induced shifts in demography if elemental ratios differed among life stages. This hypothesis was not supported. As predicted, immatures (larvae and pupae) did contain higher P concentrations than mature workers (Fig. 2), and stage structure did vary across diet treatments (Fig. 4). However, treatment differences in colony-level C : P and N : P ratios were not related to changes in stage structure, but instead were driven by the higher P concentrations in individual life stages of LSHP colonies. We must emphasize that we tested for a diet-induced linkage between stoichiometric and demographic variation using only a short-term experiment on one ant species, and we have no information about whether stage distributions reached equilibria. Nevertheless, differences in elemental ratios among life stages suggest demography may affect colony-level homeostasis in other circumstances, such as over longer time periods, through colony ontogeny, or across social insect species with different life histories.

Consistent with the GRH, colony-level mass gain decreased with colony N : P and C : P ratios. However, these relationships were rather weak because sugar scarcity increased colony-level P concentrations but did not significantly affect growth rate. These results suggest that growth rate in social colonies may depend on the balance of resources for tissue production in larvae and fuel for adults that provision and care for larvae. A research challenge is to determine the optimal mixture of adult and developing tissue (and the colony-level C : N : P stoichiometry) that maximizes growth in an ecological setting. Determining the biogeochemical signature of rapid growth in ants should elucidate mechanistic linkages among resource quality, colony life history, and the form of ecological interactions.

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