The trophic ecology of castes in harvester ant colonies

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Summary

1. The castes of social insects can vary dramatically in physiology, morphology and behaviour, and are a model of phenotypic plasticity. Dietary variation has been implicated in the evolution of castes and is involved in developmental differentiation. This study uses the elemental and isotopic composition of castes to infer the diets they consumed during larval development, when caste was determined.

2. We analysed the elemental (C : N) and isotopic ($\delta^{13}C$ and $\delta^{15}N$) composition of individuals of all castes in colonies of *Pogonomyrmex badius*, the Florida seed harvester ant, at each of two sites. Sampled herbivores and insectivores were used to calibrate measures of colony trophic position. The proportions of insects and seeds foraged by colonies correlated with inferred trophic position, strengthening the inference of diet from isotopic composition.

3. Pupae of each of the four castes (male, reproductive female or gyne, major worker and minor worker) are distinguishable based on their composition and inferred diet during the larval phase.

4. Within colonies, larval diet inferred from $\delta^{15}N$ suggests that the largest individuals assimilated more insect/protein relative to seeds. This result was consistent across colonies despite high variation in a colony's relative trophic position.

5. Carbon to nitrogen ratio (C : N) of each caste was also different, and differences were consistent across colonies and sites. While females increase in N content with increasing size, the pattern is reversed in males. This result may reflect basic life history differences (e.g. life span) between males and females. Since female reproductive potential correlates with female size in this species, the decrease in N content with increasing female caste size supports that reproductive development is N-limited, and that workers are trophically castrated due to N deprivation during larval development.

Key-words: stoichiometry, social insect, diet, polyphenism, development, stable isotopes

Introduction

In animals, nutrition during development can lead to morphological, physiological and behavioural differences as adults. Variation in diet is a major factor contributing to physical differences within and among species (Grant & Grant 2002) and is a driver of life history evolution (Stearns 1992). The castes of eusocial insects are extreme examples of alternative adult phenotypes which typically differ greatly in size, longevity, and fecundity (Hölldobler & Wilson 1990). Many authors have argued that variation in diet among individuals was fundamental to the evolution of eusociality (reviewed in Wilson 1971; Michener 1974; and Hunt 1991, 1994); when some individuals are deprived of nutrition during development they become 'trophically castrated' (Marchal 1897) and develop as non-reproducing

workers. Basing reproductive division of labour on environmental (diet/nutrition), rather than genetic factors, greatly reduces the potential for cheating genotypes and provides a mechanism by which colony level adaptive caste ratios can be maintained (Oster & Wilson 1978; Anderson, Linksvayer & Smith 2008). Understanding the proximate mechanisms underlying the evolution and maintenance of complex sociality is an essential complement to ultimate explanations, especially inclusive fitness theory, which have received much greater theoretical and empirical attention (Crozier & Pamilo 1996; Foster, Wenseleers & Ratnieks 2005; Hölldobler & Wilson 2008).

That nutritional differences lead to developmental differences between castes is not surprising, and this conclusion is supported by many empirical studies across social species (Hunt & Nalepa 1994). However, in most species the dietary differences are largely quantitative with the notable exception of the honey bees, *Apis mellifera*; honey bee larvae that develop as queens tend to receive a greater proportion of

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royal jelly than those that become workers (Haydak 1970; Michener 1974). In contrast to the extensive work on honey bees, differences between castes in diet or assimilation have received less attention in ants. This study was primarily motivated by the following two factors. First, although the queen and worker castes of the honey bee are radically different in morphology, physiology and behaviour, many species of ant have evolved a greater diversity of both form and function (as well as more extreme differences) between and within the worker and reproductive castes (Hölldobler & Wilson 1990). Thus, the potential influence of nutrition likely extends beyond queen-worker differences into worker caste morphological differences. Second, ants represent an independent evolutionary origin of eusociality from bees; comparisons across independent origins will broaden our understanding of the proximate and ultimate factors affecting caste determination (Smith *et al.* 2008a).

Seed harvester ants in the genus *Pogonomyrmex* are an emerging model system for the study of caste determination. In some species, reproductive females (gynes) and workers are produced from genetically distinct lineages co-occurring in a colony (Helms Cahan *et al.* 2002; Julian *et al.* 2002; Volny & Gordon 2002; Anderson, Linksvayer & Smith 2008). Although this system of caste determination is rare, caste determination in many ants may be influenced by genetic differences (Anderson, Linksvayer & Smith 2008; Smith *et al.* 2008a). For example, in *Pogonomyrmex badius* (Fig. 1), genetic variation exists for both caste and size, but larval nutrition and the resources available to the colony are also important (Rheindt, Strehl & Gadau 2005; Smith 2007; Smith *et al.* 2008b). *Pogonomyrmex badius* provides an ideal system for investigating the role of nutrition on caste for a number of reasons. First, it is only one of two species in the genus that has evolved multiple worker castes; worker size variation in *P. badius* is approximately double that of all other North American *Pogonomyrmex*. Therefore, it has been of particu-

lar interest because it provides insight into the evolution of novelty, worker morphological division of labour (Traniello & Beshers 1991; Ferster & Traniello 1995; Ferster, Pie, & Traniello 2006), and how a complex caste system is regulated (Tschinkel 1999; Smith 2007; Smith *et al.* 2008b). Second, *P. badius* has a broader diet than many *Pogonomyrmex* by consuming a high proportion of insects relative to seeds (Smith & Tschinkel 2005) which may facilitate the detection of differences among castes in what they have been fed during development.

Studies of trophic ecology (Peterson & Fry 1987) and ecological stoichiometry (Sterner & Elser 2002) have shed much light on the role, and flow, of basic nutritional elements (N, C, P) in communities. For example, isotope ratios of C and N provide insights into the basal C source in communities and relative trophic position of animals (DeNiro & Epstein 1978, 1981). Body size in insects is correlated with %N (Fagan *et al.* 2002). Animals feeding at low trophic levels tend to be N-limited (Kay 2002), and reproductive development in some animals is N-limited. These basic insights make the use of whole body element and isotopic ratios useful for studying how individuals within a species differ in trophic ecology as well as how these differences map onto developmental differences (Smith *et al.* 2008b). Previous studies on ants have also shown substantial trophic variation within and between species (Davidson *et al.* 2003; Kay, Rostampour & Sterner 2006; Tillberg *et al.* 2006, 2007). Therefore, social species that feed on a mixed diet source (e.g. plant and animal matter) may have sufficient variation in isotopic/elemental composition even within nests in order to address how morphological variation, such as caste differences, are affected by nutritional differences during development. Ants are holometabolous insects and thus their physical growth and development occurs only in the larval stage. Comparisons in elemental and isotopic composition at the pupal stage (non-feeding) can be used to assess how diets differed as larvae, when morphology

Fig. 1. The four castes of the Florida harvester ant (*Pogonomyrmex badius*). Clockwise from the top: reproductive female (gyne), male, major worker and minor worker. Photo courtesy of Adrian A. Smith.

became differentiated, but prior to the assimilation of adult diet (Smith *et al.* 2008b). Inferring larval nutrition using elemental analyses, however, should be interpreted with caution in the absence of direct dietary information (i.e. what individuals ate) as castes may differ in fractionation (in the case of isotopes) or respiration and N use efficiency which may affect the interpretation of results; currently, however, information on caste differences are completely lacking.

In this study we sampled all four castes produced by *P. badius* (males, gynes, major workers and minor workers) to investigate how quantitative and qualitative variation in pupal composition corresponds with caste; larval diets are, in turn, inferred from composition. Specifically, we examined elemental levels of carbon and nitrogen along with stable isotope ratios of each of those elements to compare variation within colonies (among castes), between colonies, and at different sites. The mixed seed/insect diet of *P. badius* provides enough variation, even within colonies, to infer potential differences in diets of individuals, including the relative amounts of carbohydrate and protein assimilated (C : N) and the likely origins (e.g. basal carbon source, $\delta^{13}C$, or trophic level, $\delta^{15}N$) of both C and N. A previous study, Smith *et al.* (2008b), provided preliminary evidence that female castes differed in the nutrition they received. In this study we double the sample size from the previous study and expand comparisons to differences between sexes and how dietary components correlate with both size and caste variation between and within colonies at different sites. Furthermore, we calibrate these analyses with non-ant arthropod and plant samples collected from the same sites, allowing for the calculation of relative trophic positions of colonies at each site and a comparison between ant castes and non-social arthropods in elemental composition. In all, these data shed light on the basic nutrient composition of ant castes and the effect of environmental variation in basic nutrient availability on the production of very diverse phenotypes.

Materials and methods

SAMPLING AND MEASUREMENTS

Samples were collected from two well studied populations of *P. badius*, Ant Heaven (AH) and Clear Cut (CC), both in the Apalachicola National Forest, *c.* 15 km from Tallahassee, Florida. The two sites are only separated by several km, but differ greatly in successional history. Clear Cut was clear cut in 1999 and when we sampled in 2007 it had very dense vegetation, but the majority was < 1 m tall. In contrast, AH had not been cut in several decades, had mature (> 3 m) and widely spaced long-leaf pines (*Pinus palustris*) with much exposed ground. More detailed descriptions of the sites can be found in Smith & Tschinkel (2005).

Each colony was excavated to a depth of 1 m and pupae of all castes were collected. With the exception of major worker pupae, five individual pupae of each caste (males, gynes, and minor workers) were used for analyses. Five major worker pupae were also sampled when available, but no major

worker pupae were available in colony A2, and only two and four were collected in colonies C2 and C5, respectively. Pupae were chosen for analyses because they are closed systems and represent the sum of all assimilated material, minus what was respired or excreted, from larval growth, when caste determination occurs. Collections were made in late May and early June prior to nuptial flights, and at the only time of year when pupae of all castes co-occur in the nest (Smith & Tschinkel 2006). We also collected example arthropod herbivores and predators at each site to help calibrate our dietary analyses; seeds collected from granary chambers inside each nest were collected and used as a representation of plants at each site.

All samples were killed by freezing for < 12 h and then dried in an oven at 60 °C; when not in an oven samples were maintained dry using Drierite brand desiccant. Whole dried pupae were weighed to the nearest μg on a microbalance (Metler-Toledo UMX2), and then a 1500–2200 μg sample was separated and pulverized in a sterile tin capsule. A similar sample range was used for other non-*P. badius* samples, but seeds were pulverized/homogenized using liquid N_2 and 2500–3500 μg were analysed. Samples were analysed for N and C quantity and stable isotope ratios at the Stable Isotope Facility at the University of California at Davis using a Europa Hydra 20/20 continuous flow mass spectrometer.

To investigate whether our estimate of trophic position reflected the resources currently foraged by colonies we sampled the loads of 20 foragers at each colony (with the exception of C8 which consistently had low foraging activity on our sampling days). A relationship between the relative quantities of insects/seeds collected and the trophic position (calculated from $\delta^{15}N$ levels, see below) would suggest that that our estimate of trophic position is not a brief temporal anomaly, but represents consistent differences (at least over the span of weeks; from larval diet assimilated to current foraging effort).

COMPARISONS AND STATISTICS

The relative trophic position of *P. badius* at each site was calculated as suggested by Post (2002) such that the fractionation (in $\delta^{15}N$) between trophic levels and the differences at

Table 1. Isotope and element ratios for the ants (average of all castes), seeds stored in their nests (homogenized samples from granary chambers), and herbivorous and carnivorous solitary insects collected at each site. Sample sizes are in parentheses. Variances are 95% CI from all individuals sampled per category

Site	Content	$\delta^{15}N$	$\delta^{13}C$	C : N
AH	*P. badius* (75)	3·3 ± 0·2	−25·6 ± 0·2	5·9 ± 0·3
	Seeds (4)	−1·3 ± 3·3	−23·1 ± 12·7	24·5 ± 26·8
	Herbivore (5)	2 ± 1·8	−23·5 ± 7·7	4·5 ± 0·8
	Carnivore (6)	3·6 ± 0·7	−24·1 ± 3	4·6 ± 0·8
CC	*P. badius* (77)	2·3 ± 0·2	−24·8 ± 0·3	5·7 ± 0·2
	Seeds (4)	0·5 ± 3·3	−25·3 ± 9·9	19·2 ± 19·0
	Herbivore (4)	0·5 ± 1·3	−21·4 ± 12·3	4·2 ± 0·9
	Carnivore (3)	4·9 ± 3·7	−24·3 ± 0·5	4 ± 0·6

the base of the food chain are both included in the calculation (below):

$$P.\,badius = 1 + \frac{(\delta^{15}N_{P.\,badius} - \delta^{15}N_{Base})}{\Delta_n}$$

In this equation we calculate the relative trophic position of *P. badius* as a secondary consumer; the $\delta^{15}N_{base}$ is that of the seeds collected, and Δ_n is the average enrichment between trophic levels (the average between seeds to herbivores and herbivores to predators, Table 1).

To examine whether the items currently foraged by colonies reflected the colony's trophic position (the average $\delta^{15}N$ of all pupae regardless of caste) we correlated the proportion of insects collected by foragers (of all insect and plant material) with colony $\delta^{15}N$. Variation in $\delta^{15}N$, $\delta^{13}C$ and C : N were compared across castes, sites, and colonies (nested within sites) using a MANOVA; Tukey's HSD was used for post-hoc comparisons.

Despite discrete differences in morphology and adult size, pupa size varies continuously among castes (though between castes variance is greater than within caste variance). To address how composition relates to pupa size we used an ANCOVA, also taking into account the colony of origin of individuals. In order to isolate variation among sites and colonies (which were significant in the above MANOVA) we included colony as the grouping factor in the ANCOVA; site was not used because variation among colonies includes variation inherent to the sites. Due to low within colony sampling of castes ($n = 5$), castes could not be analysed independently in this analysis. However, due to inherent developmental differences between males and females we hypothesized that diet may vary in a sex-specific manner and performed the analysis with and without males included. All female castes were pooled in the analysis to maximize variation in pupa size. In each analysis $\delta^{15}N$, $\delta^{13}C$ and C : N were dependent variables, colony was the grouping variable and pupa size ($\log_{10}$ transformed) was the covariate.

To determine the source of variation in C : N we performed regressions on each %C and %N (each standardized to vary between 0 and 1 to make slopes comparable) on dry pupal mass. Males and females were analysed separately; slopes of %C and %N were compared using a *t*-test.

Lastly, we compared how social insect colonies differ from solitary arthropods in body composition; evidence of trophic castration would be a difference between workers and other groups, but similarity between reproductives and solitary arthropods. For this analysis we compared workers (pooled minor and major workers) and reproductives (pooled gynes and males) to solitary arthropods collected at the same site (pooled insectivores and herbivores). Due to heterogeneous variances (very different sample sizes) we used colony averages for workers and reproductives, which satisfied the assumption of equal variance.

Where appropriate, means and 95% confidence intervals are reported. All analyses were performed in STATISTICA 6·0 (Statsoft 2004, StatSoft Inc., Tulsa, Oklahoma, USA).

Results

SITE AND CASTE VARIATION

Our analysis of $\delta^{15}N$ suggests that the inferred trophic position of *P. badius* is intermediate between that of an insectivore and herbivore (Table 1) at both sites although our estimates of relative trophic position varied between sites. In a relative scale where 1 = primary producer, 2 = primary consumer and 3 = secondary consumer, *P. badius* at AH were close to secondary consumers (2·88) whereas *P. badius* at CC were more similar to primary consumers (1·82). The $\delta^{13}C$ signatures of our samples were within the general range of C3 plants (C3 plants tend to range from −24 to −34‰, DeNiro & Epstein 1978) which suggests that these are the predominant base of the food web for harvester ants in these communities.

The variable composed of the three metrics of trophic ecology we measured ($\delta^{15}N$, $\delta^{13}C$, and C : N) varied significantly between castes, sites and colonies within sites, but the interaction between site and caste was not significant (Table 2). Thus, although the sites, and colonies within sites, differ in their basic chemical composition, the trophic differences among castes are similar across sites. In univariate analyses, all variables were different among castes, but only $\delta^{15}N$ differed between sites (Table 2, Fig. 2). The site × caste interaction was only significant for $\delta^{13}C$ (Table 2, Fig. 2). The interaction in $\delta^{13}C$ was driven by higher levels in the minors at only CC while $\delta^{13}C$ was constant across castes at AH (Fig. 2).

VARIATION WITHIN COLONIES AND CASTES

Each caste was readily distinguishable by pupal mass, minor workers < males < major worker < gynes.

The amount of variance explained by the ANCOVA model (when the colony x pupa size interaction term was included)

Table 2. Multivariate and univariate results for caste, colony (within site) and site differences in dietary variables

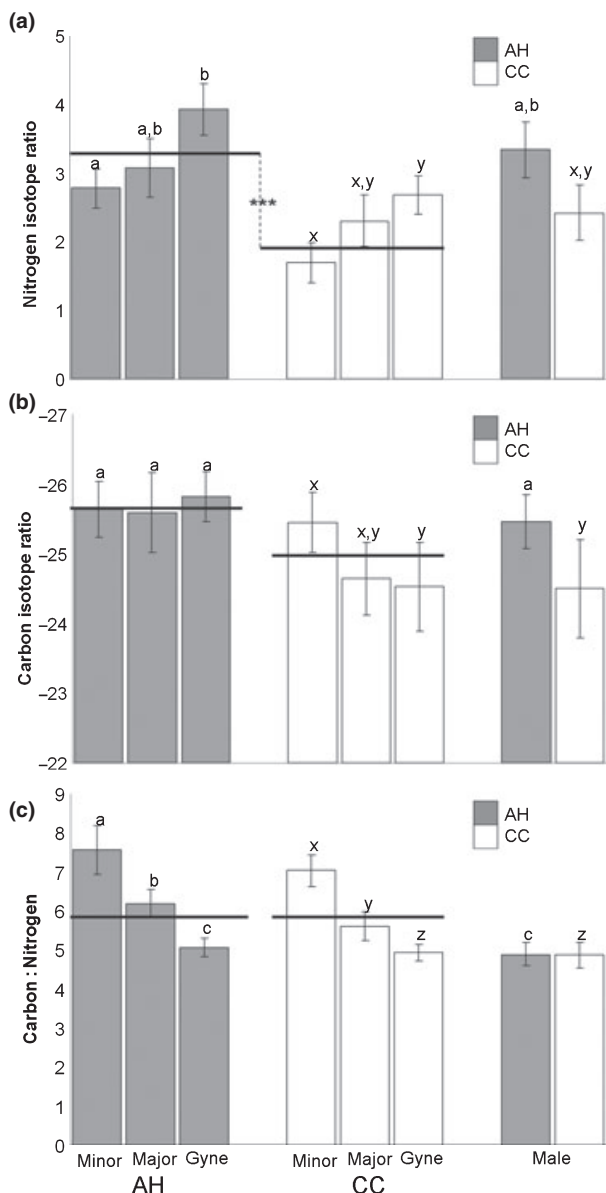| Test	Effect	Wilks λ	F	P
Multivariate	Site	0·51	55·6	< 0·0001
	Colony (Site)	0·18	31·7	< 0·0001
	Caste	0·25	38·6	< 0·0001
	Site × Caste	0·94	1·3	0·2
$\delta^{15}N$	Site	–	166·7	< 0·0001
	Colony (Site)	–	58·3	< 0·0001
	Caste	–	46·3	< 0·0001
	Site × Caste	–	1·9	0·1
$\delta^{13}C$	Site	–	2·7	0·1
	Colony (Site)	–	46·5	< 0·0001
	Caste	–	4·2	< 0·01
	Site × Caste	–	5·0	< 0·01
C : N	Site	–	0·5	0·5
	Colony (Site)	–	4·3	< 0·05
	Caste	–	73·6	< 0·0001
	Site × Caste	–	1·0	0·4

Fig. 2. Variation in the isotopic and elemental composition of castes between and within sites. Grey bars represent site AH and white bars CC. The solid horizontal lines are the site averages. Different letters represent significant within-site differences ($P < 0.05$) in a Tukey's HSD post hoc test. The asterisks in (a) indicates a site difference, $P < 0.0001$, in a univariate ANOVA. In (a) all castes had greater levels of $\delta^{15}N$ at AH compared to CC suggesting that colonies at AH fed on a relatively more insect based diet. In (b) minors differed in $\delta^{13}C$ compared to other castes only at CC resulting in a significant site x caste interaction ($P < 0.01$). In (c) all female castes were different within sites, but males were similar in composition to gynes; sites did not differ. Error bars are 95% CI from the univariate ANOVAs.

decreased for all variables when males were included. However, results were qualitatively similar for $\delta^{15}N$ and $\delta^{13}C$ with and without males, and the amount of variance explained only decreased 4% and 10% (for $\delta^{15}N$ and $\delta^{13}C$, respectively) with the inclusion of males. The inclusion of males in the analysis of C : N greatly reduced explained variance, from 66 to 41%. Thus, for $\delta^{15}N$ and $\delta^{13}C$ we present only the combined
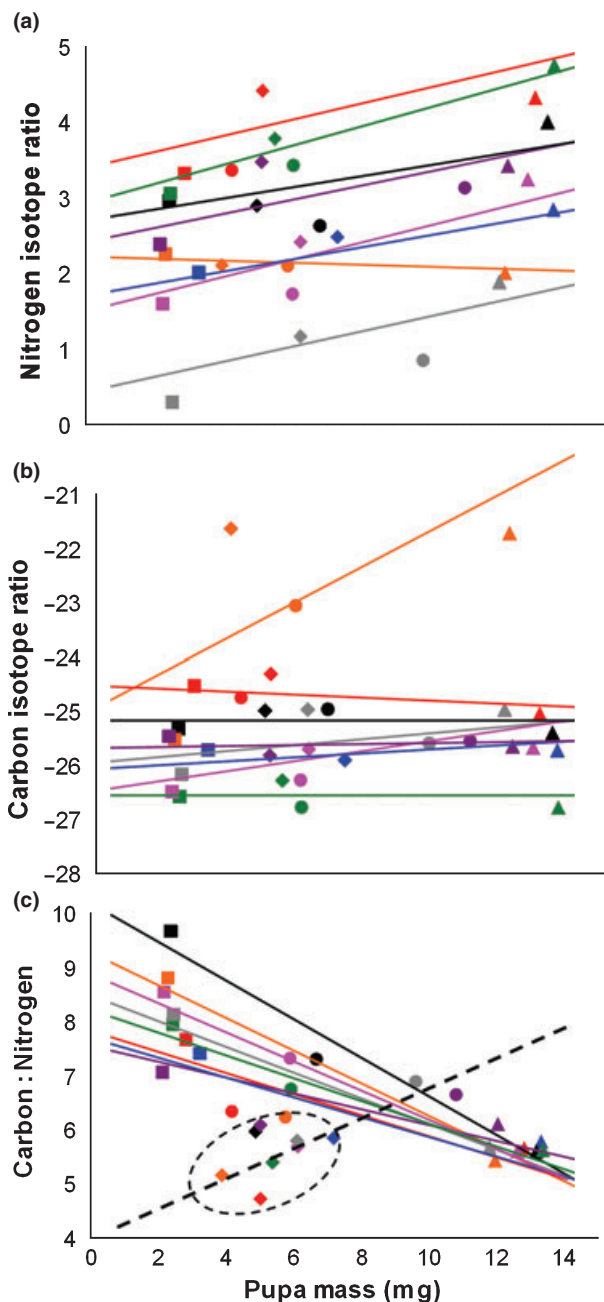
Fig. 3. The relationships between isotope and elemental ratios and variation in pupa size. Each colour represents a different colony (red = A1, blue = A2, black = A3, green = A4, pink = C2, orange = C4, purple = C5, grey = C8) and symbols are specific to castes (triangles = gynes, diamonds = males, circles = majors, squares = minors). Each symbol represents the colony average for each caste. (a) Colonies differ in their average $\delta^{15}N$, but the relationship between pupa size and $\delta^{15}N$ is parallel across colonies. (b) Pupa size does not predict $\delta^{13}C$. (c) Total C : N decreases with pupa size for females, but increases for males (dashed line); the amount of variance in C : N explained by pupa size increased greatly when slopes were fit separately for males and females. Details of slopes are in Electronic Supplement (Table S2).

model results, but include results for males and females separately for C : N. All results are available as an electronic supplement (see Table S1 in Supporting Information).

There was a significant interaction between the covariate (pupal mass) and the independent variable (colony) for all three dependent variables ($\delta^{15}N$, $\delta^{13}C$, and C : N) which indicates that slopes are not parallel. A visual inspection of these data (Fig. 3) suggested that a single colony was driving the conclusion of non-parallelism, especially for $\delta^{15}N$ and $\delta^{13}C$. Indeed, the omission of that colony resulted in parallel slopes for $\delta^{15}N$ ($F_{6,118} = 1.73$, $P > 0.1$), though not for $\delta^{13}C$ and C : N. In the analysis of $\delta^{15}N$, pupal mass is a highly significant predictor of $\delta^{15}N$ ($F_{1,124} = 93.8$, $P < 0.0001$) and colonies are significantly different ($F_{6,124} = 70.8$, $P < 0.0001$). Together, colony and pupal mass explain 79% (adjusted r^2) of the variance in $\delta^{15}N$ levels. Thus, $\delta^{15}N$ levels increase with pupal size within colonies despite colonies varying significantly in the average $\delta^{15}N$ of their pupae.

Although colonies differed in $\delta^{13}C$, pupal mass was not a significant predictor of $\delta^{13}C$ ($F_{1,118} = 0.2$, $P > 0.6$).

Pupal mass was a statistically significant predictor of C : N, but colony slopes were not parallel (Pupal Mass: $F_{1,136} = 100.0$, $P < 0.0001$). If males are excluded from the analysis of C : N, the adjusted r^2 increases from 0.41 to 0.66. Additionally, male pupal mass is positively associated with C : N (slope = 2.4, $F_{1,38} = 6.0$, $P < 0.05$, $r^2 = 0.14$) instead of negatively as is the case with female pupae (mean slope across colonies = −3.2). Full regression information of the relationship between each variable and pupal size is available by colony as a supplement (see Table S2).

As above, the regression of standardized %N and %C on pupal mass was done separately for males and females. In males, neither %N nor %C varied significantly with pupal mass ($F_{1,38} = 3.5$ and 2.1, $P > 0.05$, slopes were −0.21 vs. 0.10 for %N and %C respectively). The trend, however, was a greater decrease in %N relative to increase in %C, the direct opposite relationship as seen in females. For females %N increased and %C decreased with increasing pupal mass ($F_{1,110} = 81.6$ and 13.4, $P < 0.005$ for %N and %C respectively). The slopes in females were significantly different (females: for %N and %C, respectively, slope = 0.21 vs. −0.09, t = 12.4, d.f. = 110, $P < 0.0001$). Thus, the change per unit increase in mass resulted in a greater than doubling of %N relative to loss of %C in females.

COLONY FORAGING

There was a positive correlation between the proportion of insects (of all insect and plant material) and the average $\delta^{15}N$ of colonies ($r = 0.86$, $P = 0.03$) when one outlier colony was excluded. The same colony is an outlier with regard to both $\delta^{15}N$ and $\delta^{13}C$ (colour orange in Fig. 3).

COMPARISON TO NON-SOCIAL ARTHROPODS

The ANOVA revealed a highly significant difference between groups in C : N composition ($F_{2,39} = 27.4$, $P < 0.0001$); pair-wise comparisons indicate that reproductives and solitary arthropods did not differ from each other ($P = 0.39$), but each of those groups differed significantly from workers

($P < 0.001$). The difference between reproductives and solitary arthropods was over 5 times less than the difference of either group to workers. (means ± 95% CI: workers = 6.8 ± 0.3, reproductives = 4.9 ± 0.1, solitary arthropods = 4.6 ± 0.4); Fig. 2c illustrates the differences among castes (which were similar across both sites). Minor workers have the highest C : N, followed by major workers and then males and gynes are not different from each other and lowest in C : N.

Discussion

We show that the elemental and isotopic composition of an ant pupa corresponds well to its caste and size (Fig. 2). With double the sample size, our results corroborate earlier findings by Smith *et al.* (2008b) for female castes and provide a number of new insights: (1) the basic C : N composition of females decreases with increasing body size (due to an increase in N), but the pattern is reversed for males; (2) the caste-specific C : N is similar across colonies and sites despite a great variation in colony diets (inferred from $\delta^{15}N$); (3) the C : N of males and gynes is similar to that found in adult solitary arthropods at our sites while workers are relatively poor in N; and (4) despite large differences in the inferred sources of colony diets, larger individuals assimilated nutrition from a higher inferred trophic level within any colony. Although previous studies on ants have shown that colonies fed different amounts of protein differ in their caste ratios (minors vs. majors or workers vs. gynes, e.g. Goetsch 1937; Smith 1942; Passera 1974; Bono & Heithaus 2002; Smith 2007), dietary differences among individuals have remained enigmatic. Our study demonstrates the power of basic elemental analyses for inferring the diets of developing individuals, but manipulative experiments are required to demonstrate the causality of patterns we report. If applied to a greater range of species, especially coupled with dietary manipulations, this basic methodology has the capacity to greatly expand our knowledge of the role of nutrition as a regulator of social organization (e.g. as in caste determination) and how nutritional variation may have influenced the evolution of sociality (e.g. the formation of a reproductive division of labour).

VARIATION IN AVAILABLE RESOURCES

When all castes from all colonies are pooled, the variation in the $\delta^{15}N$ is large, spanning two trophic levels, from seeds to carnivorous insects (Fig. 3a, Table 1). This high amount of variation appears to reflect the availability of different food items at each site. A collection of foraged material at colonies was correlated with the colony's average $\delta^{15}N$, supporting that the sites differ in insect availability, and that these differences are consistent at least over the course of weeks (nutrition assimilated by larvae compared to current foraging effort). Although our measurements are consistent with the general trend of increased $\delta^{15}N$ with trophic level (DeNiro & Epstein 1981), since we did not directly calibrate our measure-

ments from the diets of individuals the linking of isotopic data to larval diets is inferential.

Despite variation across sites and colonies, we infer that the diet of larger castes is from a higher trophic level than that of the smaller castes. Intriguingly, the data suggest that within colony dynamics play an important role in the nutrition supplied to individuals developing as each caste; not only do larger castes differ from smaller castes, but the differences are much stronger when their colony of origin is accounted for. The higher $\delta^{15}N$ of larger castes may actually represent an increase in consumption of trophic (nutritional) eggs rather than foraged insects, but our data are insufficient to distinguish the source of N. *P. badius* likely distribute energy through the nest via trophic egg production as they lack the ability to exchange food through trophallaxis (liquid regurgitation, Eisner & Wilson 1958; Smith 2007).

The role of carbon sources (as judged by $\delta^{13}C$) was much less clear in our data. While at one site, AH, there were no differences in $\delta^{13}C$ among castes, at CC the minor workers had more negative values than the larger castes. $\delta^{13}C$ tends to increase with trophic position (*c*.1‰, DeNiro & Epstein 1978) which may explain the decreased values in larger individuals at CC, but it is unclear why this relationship would be absent at AH. The difference between sites in the caste relationships of $\delta^{13}C$ is more likely a change in dietary source, perhaps with a greater reliance on plants with C_4 photosynthesis; the vegetation did differ quite dramatically between sites due to the more recent clear-cutting of site CC.

NUTRITIONAL REGULATION OF CASTES

A strong effect of nutrition on caste determination likely increases the ability of the colony to respond to environmental variation, such as a decrease or change in the food supply. If food is in short supply the workers can modify how food is allocated to castes. For example, when experimentally starved, *P. badius* colonies increased investment in growth relative to reproduction compared to fed colonies (Smith 2007). Thus, when food and limiting nutrients are in short supply colonies likely modify how these are allocated to different castes. If nutritional inputs are the controllers of developmental switches, then colonies can flexibly, and adaptively, adjust caste production.

The fact that there was a difference between males and females in their C : N by body size relationship is not surprising given that their growth trajectories diverge early in development (egg stage). There is continuous variation in ovary size (ovariole number) across all female castes in *P. badius* and head size predicts 75% of the variation in ovariole number (Smith *et al*. 2007). The decrease in C : N with increasing size is due to increasing N content more than decreasing C. Furthermore, the parallel relationships of ovary size and increasing N content with individual size suggest that reproductive development is N-limited. Indeed, N-limitation of reproduction has been documented in termites (Brent & Traniello 2002), and N-limitation may have been important in the transition to eusociality in the Isoptera (Nalepa 1994)

as has also been predicted for hymenopteran social insects (Marchal 1897; Hunt & Nalepa 1994). Furthermore, protein is generally limiting for oogenesis in animals (Wheeler 1996). Further study, however, is needed to establish causality in this relationship; although suggestive, our results cannot distinguish whether N is a cue regulating a developmental switch or downstream of the differentiation of caste developmental pathways. The female castes of *P. badius*, like most ants, cannot be differentiated with high certainty until the pupal stage, thus making it difficult to determine when developmental trajectories diverge. The genetic caste determination systems of some ants (Anderson, Linksvayer & Smith 2008) may be very useful as models for caste development because castes can be determined with genetic markers even when undifferentiated.

Although female reproductive development appears N limited, males incorporate less N per unit increase in pupa size (Fig. 3c). As in most eusocial Hymenoptera, *P. badius* males fulfil a single function, mating. A male's life is very fleeting once mating is terminated. Thus, increasing C : N with increased pupa size may serve as short term resource storage which is needed for flight.

THE BENEFITS OF INDIVIDUAL SIZE

Although it is not possible with our data to differentiate between whether larger individuals solicit more N-rich food (e.g. insects, trophic eggs) and/or whether more N-rich food leads to increased growth, these results strongly suggest that larger individuals consume food from a higher trophic level (more insects relative to seeds), which in turn increases the proportion of N in their diet. Large individuals in colonies feeding at a relatively lower trophic position may need to eat more than their counterparts in colonies feeding at a higher level; this type of compensation has been observed in other insects (Taylor 1989). This would imply that colonies feeding at a higher trophic level are more efficient at nutritional allocation to the different castes and waste less effort in eating nutritionally inferior food.

As colonies are very genetically diverse (on average the queen mates with 20 males, Smith *et al*. 2008b) there is the potential for conflict over which individuals develop as gynes (Ratnieks, Foster & Wenseleers 2006), and which gynes grow the largest (Smith *et al*. 2008b). There is a rather large queen-worker size difference in *P. badius*, making it less likely that developing larvae can directly influence their caste fate (Bourke & Ratnieks 1999; Wenseleers, Ratnieks & Billen 2003), and patriline only weakly predicts caste; most patrilines produced all castes (Smith *et al*. 2008b). Larvae, however, may have the ability to influence their size after caste has been determined, which may still result in fitness differences among genotypes of a colony; indeed, genetic variation is a statistically significant contributor to female size in *P. badius* (Smith *et al*. 2008b). Gyne size is documented to be under natural selection during the founding stage of some harvester ants (Wiernasz & Cole 2003). Thus, developing larvae that are better able to gain high quality nutrition will likely have a fitness advantage. Similarly, workers are capable of reproduction in

P. badius and reproduce in the absence of the queen (Smith *et al.* 2007). As larger workers have larger ovaries even larvae that are on the worker developmental pathway may gain potential fitness (via male production when the colony is orphaned) by increasing N consumption. While the self-biasing of size and caste may produce conflict, colonies might also benefit by producing variation in gyne and worker size. Size variation may be beneficial through effects on worker division of labour (Oster & Wilson 1978) and when size specific mortality among founding queens is highly variable (i.e. under good conditions even small queens may be successful). Sexual selection on male body size has also been documented in harvester ants (Davidson 1982; Abell *et al.* 1999); males that are fed better may fare better in gaining matings and increasing inseminate volume, which in turn will increase both individual and colony fitness.

A BROADER PERSPECTIVE ON CASTE DETERMINATION

Males and females have similar C : N, and their C : N is similar to the random solitary insects sampled in these populations (Table 1, Figs 2 and 3c); the average C : N of workers was 40% greater than that of reproductives. This result supports the intuitive and historic notion that workers are trophically castrated (Marchal 1897; reviewed by Hunt 1991, 1994). Furthermore, castration comes in two basic forms, moderate (major workers) and extreme (minor workers), suggesting that diet is likely a regulator of both queen-worker and worker-worker development.

Qualitative differences in diet may be due to temporal variation in the food supply or a change in the diet received by larvae developing early vs. late in the year. The production schedule in some ants is regulated by maternal effects; after an environmental cue (e.g. overwintering) the queen may increase resources in each egg, including juvenile hormone levels (DeMenten *et al.* 2005; Schwander *et al.* 2008). The initiation of reproduction appears highly synchronized in *P. badius* (Smith & Tschinkel 2006), which may be driven by temperature, perhaps in combination with maternal effects. Although increased egg quality may contribute to a biasing of caste determination, our results suggest that larval diet is also important. Clearly, the biasing of caste at the egg stage (through genetic or maternal effects) is insufficient for caste determination given the large size and morphological differences among female castes in *P. badius*. Larger castes must receive more nutrition and increased reproductive development may require a different type of nutrition.

In summary, many factors contribute to the regulation of caste production and the determination of a developing individual's caste fate and final adult size. Nutrition is a likely master regulator of caste production, influencing differential gene expression and endocrine responses (Anderson, Linksvayer & Smith 2008; Smith *et al.* 2008a); however, much work is needed to understand the interplay between the nutrient sensing pathways of individuals, nutrient distribution within colonies, and nutrient availability in the environment.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1. Complete ANCOVA results.

Table S2. Regression analyses by colony.

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