Diet composition does not affect ant colony tempo

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Summary

1. Resource availability can influence the structure of animal communities by mediating competitive interactions. An underappreciated aspect of resource ecology is how functional traits are built from particular sets of nutrients and thus are underexpressed when those nutrients are scarce.

2. One idea linking resource availability to competition is the metabolic fuel hypothesis, which posits that access to carbohydrate-rich resources favours high activity rates that increase competitive prowess. One prediction of this hypothesis is that an increase in dietary carbohydrate richness will lead to an increase in consumer metabolic rate (MR). An alternative prediction is that carbohydrate-rich diets will affect biochemical storage.

3. We tested these predictions by examining the effect of dietary protein : carbohydrate (P : C) ratio on the feeding behaviour, demography, physiology and MR of colonies of *Ectatomma ruidum*, a common tropical ant. We found that rearing colonies for 6 weeks on different P : C diets altered colony demography and worker storage biochemistry, but higher carbohydrate intake did not result in an increase in whole-colony MR.

4. Our results suggest that dietary carbohydrates may increase colony dominance through changes in colony composition and individual worker longevity rather than changes in worker activity rates. More generally, they illustrate how the identity of scarce nutrients can have particular effects on traits that impact ecological interactions.

Key-words: Barro Colorado Island, brown food web, carbohydrates, *Ectatomma*, Geometric Framework, metabolic rate, nutrient balance, nutritional ecology, protein

Introduction

The study of nutrition provides a context for understanding the mechanisms by which resources structure animal communities (Raubenheimer, Simpson & Mayntz 2009; Simpson *et al.* 2010). Nutrition can affect community structure if consumers select habitats and selectively forage to meet nutritional demands (Simpson *et al.* 2004). Nutrition can also structure communities if nutrient access affects the relative fitness costs and benefits of traits that require distinct nutritional mixtures (Kay *et al.* 2005). A key step in building links between nutrition and communities is clarifying how nutritional challenges affect the expression of ecologically relevant traits.

Relationships between nutrition and trait expression are a central focus of the Geometric Framework (GF), a state-space modelling approach designed to clarify how consumers respond to nutritional trade-offs (Behmer 2009; Raubenheimer, Simpson & Mayntz 2009). A focal point in GF is the distinct and interactive roles of dietary components (macronutrients, minerals and salts, toxins) on consumer physiology and behaviour. Numerous GF studies (e.g. Behmer & Joern 2008; Maklakov *et al.* 2008) have focused on the interplay between dietary protein (P) and carbohydrates (C) because these macronutrients play important roles in animal nutrition and P : C content varies among foods. Food P : C content can affect feeding behaviour when consumers selectively forage on nutritionally imbalanced foods, and diet P : C content can influence the expression of traits that are differentially affected by protein and carbohydrate scarcity.

The ecological importance of P : C balance has been a recent focus of research on ants and other social insects (Kay 2004; Dussutour & Simpson 2009; Ness, Morris & Bronstein 2009; Cook *et al.* 2010; Kay *et al.* 2010; Pirk *et al.* 2010). Many social insects collect resources that differ in P : C content (e.g. nectar vs. invertebrates). Moreover, protein and carbohydrates serve distinct nutritional needs within colonies (Blüthgen & Feldhaar 2010), as workers shunt protein to queens and larvae but retain carbohydrates (Weeks *et al.* 2004). The metabolic fuel hypothesis (MFH) links P : C balance in food and consumer behaviour. It posits that greater dietary richness of carbohydrates or other

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metabolic fuels favours investment in worker activity and aggression (Davidson 1997; Kay *et al.* 2010). The MFH has successfully predicted associations between dietary P : C and worker activity (Grover *et al.* 2007) and general associations between nectar availability and dominant ant abundance and activity (Davidson *et al.* 2003; Gibb & Cunningham 2009); however, the mechanism underlying these associations and their generality is not yet clear.

A central prediction of the MFH is that an increase in the proportion of metabolic fuel in the diet leads to a higher worker activity rate, or tempo. This prediction is consistent with the results from studies on solitary organisms, showing increased metabolic rate (MR) on low-protein diets (Zanotto, Simpson & Raubenheimer 1993; Trier & Mattson 2003). However, all or some fraction of dietary surpluses of carbohydrates and lipids can instead be used to increase fat storage (Warbrick-Smith *et al.* 2006) in lieu of increased energy expenditure (Jensen *et al.* 2010), but the extent to which this occurs is not well documented. For ants, there is some evidence that worker activity and aggression can increase in colonies fed high-carbohydrate (low P : C) diets (Grover *et al.* 2007). However, Kay *et al.* (2010) showed that an increase in territorial aggression for ant colonies on low P : C diets was not linked to difference in worker behaviour, but instead stemmed from colony size differences associated with lower worker mortality rates on low P : C diets. Controlled studies on the MFH have focused on an invasive species (the Argentine ant *Linepithema humile*), so the general importance of this hypothesis is not known.

Here, we examine the effect of dietary P : C ratio on the feeding behaviour, demography, physiology and MR of colonies of *Ectatomma ruidum*, a common ground-nesting ant in Neotropical forests (Fig. 1). Several recent studies have demonstrated the importance of dietary P : C ratio on social insect life history and behaviour (Dussutour & Simpson 2009; Altaye *et al.* 2010; Cook *et al.* 2010, 2011; Pirk *et al.* 2010), but no previous study has tested for associations between diet macronutrient composition and ant MR. We focus on *E. ruidum* because (i) it likely experiences variable dietary P : C ratios because it collects arthropods and opportunisti-

cally feeds on nectar from low vegetation and fruit falls (Franz & Wcislo 2003) and (ii) it is a widespread, abundant and an ecologically important component of understorey ant communities in the Neotropics. In addition, a recent experimental study showed that *E. ruidum* colonies can selectively forage to balance P : C intake (Cook & Behmer 2010). We use MR as a measure of tempo (see e.g. Hurlbert, Ballantyne & Powell 2008) because it is the most integrative and quantifiable measure of energy use (Schmidt-Nielsen 1997), and it is generally associated with energy output differences across individuals within species (Biro & Stamps 2010). We use whole-colony rather than individual worker MR because the colony is the main level of selection in ants and social context can strongly affect ant MR (Shik 2010; Waters *et al.* 2010). Evidence from solitary organisms suggests that dietary carbohydrate imbalance can affect MR (Hessen *et al.* 2004; Jeyasingh 2007), but the generality of this effect and the extent that it occurs in social taxa is not clear.

Our main prediction is that an increase in colony acquisition of carbohydrates vs. protein will lead to higher whole-colony MR. Such a response would provide a mechanism by which colonies could opportunistically use carbohydrate resources to increase behavioural dominance. In contrast, more lipid storage on low P : C diets and more storage of uric acid (a nitrogen-rich waste product) on high P : C diets would have functional consequences for starvation resistance and worker longevity.

Materials and methods

We manipulated diets of captive *E. ruidum* colonies in June–July 2010 on Barro Colorado Island (BCI), a lowland, seasonally wet forest in Lake Gatun of the Panama Canal (Leigh, Rand & Windsor 1996), where this species is a numerically dominant ground-nesting ant. Colonies of this monomorphic species nest in soil cavities within 1 m of the surface; single colonies can be polydomous (inhabiting multiple adjacent nests) and polygynous (containing multiple queens) at this site (A. Kay, pers. obs.). *Ectatomma ruidum* nests have a single entrance – a well-defined, *c.* 0·5-cm diameter opening – and contain *c.* 10–150 workers. We excavated 26 colonies and isolated all nest material. Fourteen colonies had at least one queen, twelve colonies were queenless. We reduced queen number to one in multiple-queen colonies and used queen presence as a random factor in all analyses. We measured the initial wet mass of each colony to the nearest microgram and enumerated larvae, pupae and workers. We maintained colonies in circular containers (18 × 8 cm) whose rims we lined with Fluon® (Northern Products Incorporated, Woonsocket, Rhode Island, USA). In each container, we placed two nest chambers (glass test tubes: 20 × 100 mm) half full of water, stopped with cotton and covered with foil. We kept colonies on tables in a covered shelter on BCI in which colonies were exposed to ambient understorey light, temperature and humidity. We covered nest containers with mesh to keep out other invertebrates.

We assigned colonies (*n* = 14 with a queen, *n* = 12 without a queen) to one of two synthetic diet treatments that differed in protein : carbohydrate ratio (P : C). Foods in each experiment were made from modifications of the diet described in Dussutour & Simpson (2008). Foods contained three protein sources (dried egg, calcium caseinate and whey protein), sucrose, methyl 4-hydroxybenzoate (to

Fig. 1. An *Ectatomma ruidum* worker. Photograph by Benoit Guénard.

prevent fungal growth) and VanderZant's vitamin mixture dissolved in an agar (Table S1, Supporting Information). Treatment P : C ratios were 3P : 1C or 1P : 3C; these ratios span the P : C ratio selected by *E. ruidum* in the field (Cook & Behmer 2010). Both foods contained 100 g L^{-1} of protein + carbohydrate.

We fed and quantified food intake for high and low P : C colonies over 42 days. We provided each colony with *c.* 1 g of agar gel every 2 days. We measured food intake at the end of each 2-day period using food dry mass loss, which we estimated using dry mass : wet mass ratio in control food blocks (four per food type per feeding). To estimate food intake per worker, we counted the number of workers in each colony at day 14, 28 and at the end of the experiment (day 42). We estimated worker number at each feeding period by assuming linear rate of change in worker number between census periods.

After 42 days, we measured whole-colony MR. For MR work, we transferred colonies to polyurethane cylinders 1 day before measurements. We then used constant volume respirometry (Sable Systems International, Las Vegas, NV, USA) to measure and record microlitres CO_2 release per hour (VCO_2), averaged over five hourly measurements following a 1-hr acclimation period. Data for each colony were standardized by measuring the empty chamber VCO_2 after ants had been removed following colony measurements. Each data point was converted to the value at 25 °C assuming a Q_{10} of 2 (as per Lighton 2008). However, minimal temperature corrections were needed because the mean (± 1 SD) of 70 hourly temperature measurements was 21·6 $\pm$ 0·7 °C (for additional information on MR methods, see Appendix S1, Supporting Information). Following MR measurements, we froze colonies at −20 °C for 2 h. We then vacuum-dried the colonies for 48 h and weighed components to the nearest microgram.

After freezing colonies, we measured several worker biochemical traits using vacuum-dried samples. We measured carbon (C) and nitrogen (N) content in whole workers using a Flash EA1112 CN analyzer (ThermoQuest, Milan, Italy) that completely combusts samples; we used aspartic acid as a standard. We determined total lipid content in individual workers using a phosphovanillin reagent as in Grover *et al.* (2007); we used a 70 : 30 mixture of glycerol trioleate : glycerol tripalmitate as a standard. We determined uric acid content in using an Amplex® Red Uric Acid Assay Kit (Life Technologies, Carlsbad, California, USA). We first homogenized freeze-dried ants to a fine powder, then extracted uric acid in 50 mM Tris–HCl (pH 7·4) using a freeze-thaw cycle to disrupt tissue. We incubated samples with Amplex® Red reagent/Horseradish peroxidase/Uricase solution for 30 min, and then measured fluorescence intensity with a Tecan Genios Fluorescent Microplate Reader using a 535 nm excitation filter and emission detection at 595 nm. We compared fluorescence intensity to a standard curve. We used two to three workers per colony for each biochemical assay; mean value per colony was used in statistical analyses.

We used JMP 8.0.1 (SAS Institute, Cary, North Carolina, USA) for statistical analyses. We assessed the effects of diet, queen presence and diet-by-queen interaction over time on food intake and worker mortality using MANOVA. We assessed the effects of these factors on worker traits using ANOVA. For colony MR, we included colony dry mass as a covariate in the analysis of log$_{10}$-transformed data. We only include analysis of a main effects model for colony MR as no interactions in the full model were significant.

Results

Total food intake did not differ between diet treatments (diet: $F_{1,22} = 0·645$, $P = 0·431$). As a result, protein intake was

significantly higher (diet: $F_{1,22} = 54·110$, $P < 0·001$) and carbohydrate intake was significantly lower (diet: $F_{1,22} = 76·238$, $P < 0·001$) for colonies on the high P : C (high-protein) diet compared to those on a low P : C (high-carbohydrate) diet (Fig. 2). Colonies with queens consumed more food than did those without queens (main effect of queen: $F_{1,22} = 6·637$, $P = 0·017$). There was no significant diet-by-queen interaction on total intake of food ($F_{1,22} = 0·184$, $P = 0·672$), protein ($F_{1,22} = 1·768$, $P = 0·197$) or carbohydrates ($F_{1,22} = 0·914$, $P = 0·350$).

Demographic changes differed predictably between treatments (Fig. 3). Colonies on the high P : C diet lost more workers + pupae ($F_{1,22} = 2·23$, $P = 0·036$) but suffered less decline in larval wet mass than did colonies on the low P : C diet ($F_{1,22} = 2·60$, $P = 0·016$). The change in number of workers + pupae did not vary with queen presence ($F_{1,22} = 0·31$, $P = 0·758$), but, unsurprisingly, colonies without queens suffered greater decline in larval wet mass ($F_{1,22} = 4·26$, $P < 0·001$), particularly those on the high P : C diet (diet-by-queen interaction: $F_{1,22} = 2·10$, $P = 0·048$).

Diet significantly affected biochemical storage in workers (Fig. 4). Workers from colonies on the high P : C diet had significantly lower C : N ratios (molar ratios: 4·81 $\pm$ 0·64 (1P : 3C) vs. 5·51 $\pm$ 1·37 (3P : 1C); $F_{1,22} = 5·19$, $P < 0·001$), lower lipid content (Fig. 4; $F_{1,18} = 6·00$, $P < 0·001$) and higher uric acid content (Fig. 4; $F_{1,22} = 4·630$, $P < 0·001$) than did workers from colonies on the low P : C diet. Queen presence did not significantly affect any physiological measure, although there was a significant diet-by-queen interaction for C : N ratio as it differed less with queen presence on the high P : C diet (molar ratio :
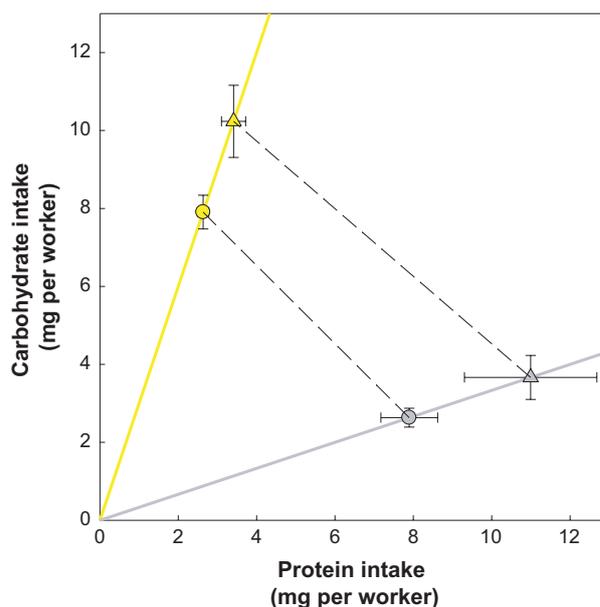

Fig. 2. Cumulative protein intake vs. carbohydrate intake (means $\pm$ 1 SE) for *Ectatomma ruidum* queenright (triangles) ($n = 12$) and queenless (circles) ($n = 14$) colonies reared on either a 1P : 3C diet (yellow symbols) or a 3P : 1C diet (grey symbols) for 42 days.
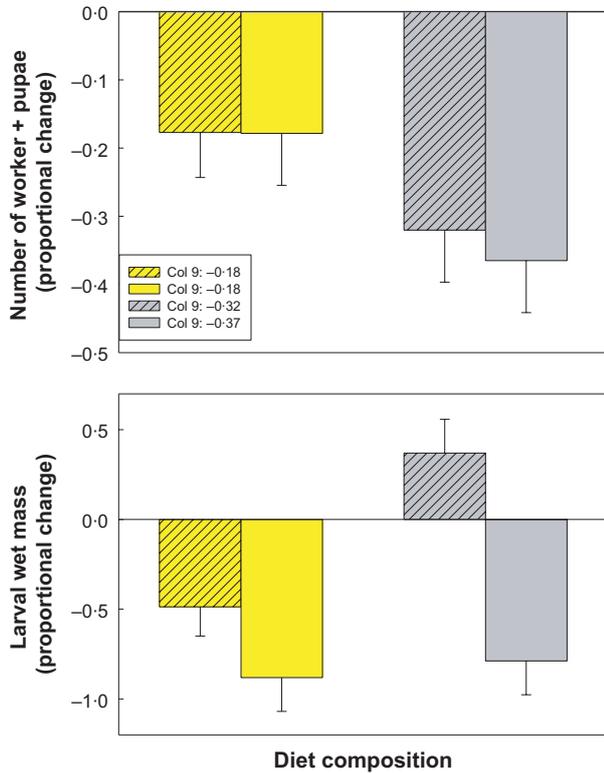
Fig. 3. Changes in demographic composition for *Ectatomma ruidum* queenright (hashed bars) (*n* = 12) and queenless (solid bars) (*n* = 14) colonies reared on either a 1P : 3C diet (yellow bars) or a 3P : 1C diet (grey bars) for 42 days. Data are means ± 1 SE. Upper panel shows proportional changes in the number of workers and pupae over the time of the experiment; lower panel shows proportional changes in larvae wet mass.
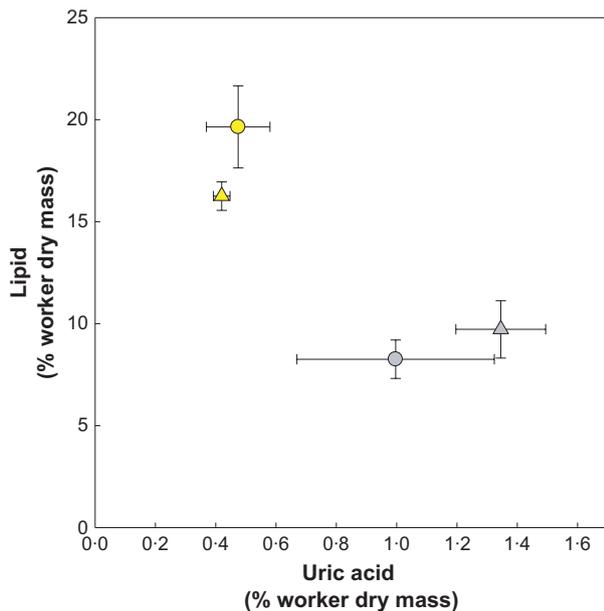

Fig. 4. Lipid content vs. uric content (% dry mass, means ± 1 SE) for *Ectatomma ruidum* queenright (triangles) (*n* = 12) and queenless (circles) (*n* = 14) colonies reared on either a 1P : 3C diet (yellow symbols) or a 3P : 1C diet (grey symbols) for 42 days.

colonies with queen = 4·875 vs. no queen = 4·755) than on the low P : C diet (5·837 vs. 5·269).

In contrast, the high-carbohydrate diet did not generate higher whole-colony MRs (Fig. 5). Across all 26 colonies, which ranged in dry mass from 55 to 708 mg, temperature-corrected MR scaled as 0·29 *colony mass*$^{0·91 \pm 0·12}$, with a log–log slope that did not differ significantly from isometry (*F*-test $b = 1$; $F_{1,24} = 2·02$, $P = 0·170$). Colony MR was not affected by either diet (Fig. 5; $F_{1,22} = 0·40$, $P = 0·695$) or queen presence (Fig. 5; $F_{1,22} = 0·53$, $P = 0·600$).

Discussion

We tested whether ant colony tempo and biochemical storage responded to changing dietary P : C ratio using a laboratory study of *Ectatomma ruidum*, a common tropical ant. While differences in macronutrient uptake altered colony demography and worker storage biochemistry, higher carbohydrate intake failed to generate increases in whole-colony MR. If dietary carbohydrates increase colony dominance as predicted (Davidson 1997), our results suggest that these phenotypic changes emerge from changes in colony composition rather than changes in individual worker activity rates.

The lack of change in colony MR to nutritional imbalances (Fig. 4) was unexpected given previous studies (on solitary insects) showing an increase in MR on lower P : C diets – a response interpreted as a mechanism to maintain nutrient balance by voiding excess energy (Zanotto, Simpson & Raubenheimer 1993; Zanotto *et al.* 1997). Similarly, Grover *et al.* (2007) found limited evidence using behavioural assays that individual workers from colonies of the Argentine ant, *Linepithema humile,* reared on lower P : C (carbohydrate-rich) diets have higher activity and aggression rates. In a follow-up study, Kay *et al.* (2010) found that Argentine ant colonies show more territorial aggression when reared on lower P : C diets, but this increase stemmed from larger worker populations associated with lower worker mortality
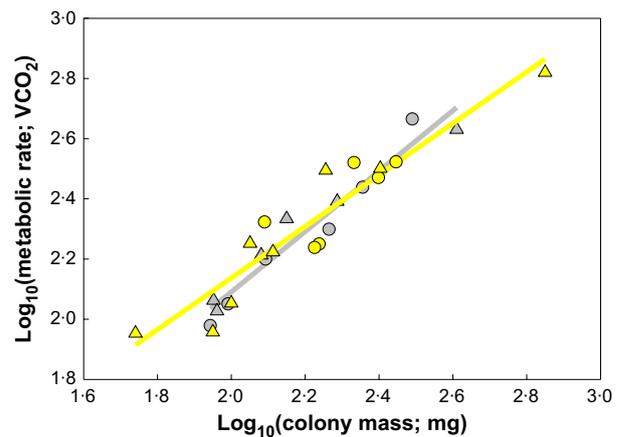

Fig. 5. Log$_{10}$–log$_{10}$ plot of metabolic rate (VCO$_2$) vs. colony dry mass for *Ectatomma ruidum* queenright (triangles) (*n* = 12) and queenless (circles) (*n* = 14) colonies reared on either a 1P : 3C diet (yellow symbols) or a 3P : 1C diet (grey symbols) for 42 days.

rates rather than from changes in individual worker behaviour. These results, and the results from the current study showing shifts towards lipid storage rather than increased MR on a lower P : C diet, suggest that observed associations between carbohydrate access and behavioural dominance in ants may result primarily from affects on worker population sizes rather than changes in individual activity rates.

Studies on solitary species suggest that insects adapted to energy-poor diets may often store rather than rapidly metabolize energy from high-carbohydrate diets (Warbrick-Smith *et al.* 2006). Similar to our study, Jensen *et al.* (2010) found that wolf spiders fed a high lipid : protein diet had higher lipid : protein body composition and extended survival but did not have increased MR relative to spiders on a low lipid : protein diet. Jensen *et al.* (2010) suggested that a storage response rather than a MR response was an adaptation to episodic food availability associated with an opportunistic predator strategy. The response by *E. ruidum* colonies to store rather than metabolize excess carbohydrates may similarly be a response to opportunistic resource availability. *Ectatomma ruidum* colonies inhabit tropical rain forest understorey habitat where flowering and fruit falls, and associated nectar availability (see e.g. Yanoviak & Kaspari 2000), may be staggered in time and space, leading to droughts of carbohydrate availability. Converting these nectar resources into lipid may allow colonies to maintain consistently large worker populations in the face of resource fluctuations.

The importance of uric acid accumulation in *E. ruidum* colonies on the high P : C diet is less certain (Fig. 3), although it may be connected to colony demographic changes (Fig. 2). Uric acid is a waste product and its accumulation is a likely indicator of physiological stress (O'Donnell 2008). Studies on solitary species have suggested that uric acid accumulation contributes to increase mortality on high-protein diets (e.g. Lee *et al.* 2008), although the exact mechanism linking diet P : C ratio, uric acid accumulation and mortality still need to be determined. Interestingly, higher mortality rates are also seen in honeybees fed high P : C diets (Pirk *et al.* 2010), suggesting worker intolerance to high-protein diets may be a general feature in social hymenoptera. Uric acid can serve as a nitrogen storage in some solitary insects (e.g. Hongoh & Ishikawa 1997), but other storage proteins are likely more important in ants (Wheeler & Martinez 1995).

A few caveats are in order when interpreting our MR results. First, colonies may have used food processing mechanisms to reduce or eliminate dietary nutrient imbalance before consumption. Recent studies on ants have shown that colonies feeding on nutritionally imbalanced agar-based diets can selectively extract carbohydrates from pieces of agar that have been returned to the nest (Dussutour & Simpson 2009; Cook *et al.* 2010). If *E. ruidum* colonies selectively extracted substantial nutrients from their agar food, the P : C ratio actually consumed by colonies may not have differed between treatments and thus would not be expected to affect colony MR. However, the demographic and physiological responses associated with diet suggest that P : C intake did in fact differ significantly between treatments. Second, the

lack of MR response may have resulted from the limitations of laboratory confinement. Colonies in nature may use carbohydrates to increase MR on demand, for example when they can invest in foraging or territorial behaviours that increase fitness. Future studies should include outlets for such behaviours. Third, associations between diet P : C ratio and colony MR may be more likely in ant species that are more reliant on nectar. Stable isotope data (Davidson *et al.* 2003; Russell *et al.* 2009) suggest that *Ectatomma* rely less on plant material such as nectar than many other ant species. We predict that more herbivorous ants with regular supplies of sugar (e.g. canopy ants, or those from light-filled forest gaps that tend coccids or harvest plant exudates, Yanoviak & Kaspari 2000) will be less likely to store, and more likely to metabolize, sugar windfalls. This prediction could explain our previous result that showed increased activity and aggression for Argentine ant workers from colonies reared on low P : C diets (Grover *et al.* 2007). Finally, it is worth noting that all colonies lost mass during the experiment, suggesting that there may have been a deficiency in the synthetic diet that interacted with treatment effects. This diet has been used in several recent studies (e.g. Dussutour & Simpson 2008, 2009; Cook *et al.* 2010, 2011), but it is still unclear how well this diet promotes growth relative to other synthetic or natural diets across a range of ant species. Regardless, in our study, colonies with queens on the low P : C diet did show an increase in total larval mass during the experiment (Fig. 2), suggesting colony growth would have occurred in this treatment in a longer experiment.

Although we found no support for an association between carbohydrate access and ant tempo in *Ectatomma ruidum*, dietary carbohydrate content can still increase ant behavioural dominance through changes in colony demography and worker behaviour. Results in this study and others (Kay, Rostampour & Sterner 2006; Grover *et al.* 2007; Dussutour & Simpson 2009; Cook *et al.* 2010; Kay *et al.* 2010; Pirk *et al.* 2010) suggest that increased dietary carbohydrate content increases worker survivorship. All else equal, higher worker survivorship will increase colony size, a key determinant of competitive prowess in ant communities (Palmer 2004). In addition, an increase in carbohydrate availability may increase colony demand for protein resources (Kay 2004) and worker aggression towards protein-rich prey (Ness, Morris & Bronstein 2009). These results suggest that carbohydrate availability may be an important mediator of ant interactions, and, more generally, that the identity of scarce nutrients can have particular effects on traits that impact ecological interactions.

Acknowledgements

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Supporting Information

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

Appendix S1. Detailed respirometry methods.
Table S1. Components of experimental diets used in this study.