RESEARCH ARTICLE

Macronutrient regulation in the Rasberry crazy ant (*Nylanderia* sp. nr. *pubens*)

S. C. Cook · R. A. Wynalda · R. E. Gold · S. T. Behmer

Abstract Animals grow and optimize performance when they collect foods in amounts and ratios that best meet their species-specific nutritional requirements. For eusocial organisms like ants, where only a small fraction of the colony members collect food, increasing evidence demonstrates that strong macronutrient regulation occurs at the colony level. Here, we explored regulation of protein and carbohydrate (p:c) intake in the Rasberry crazy ant, *Nylanderia* sp. nr. *puben.* We did this using dry artificial foods (14–42% total macronutrient content) having a range of fixed p:c ratios in a series of choice and no-choice laboratory experiments and used worker mortality to gauge colony-level costs associated with active nutrient regulation. Choice experiments revealed that colonies preferred carbohydrate-rich foods and self-selected a diet having a p:c ratio $\sim$1:2. No-choice experiments demonstrated that food p:c ratio only moderately affected worker food collection behavior, likely because colonies regulated the intake of only the non-limiting nutrients. Absolute worker mortality was generally high, but lowest in colonies feeding on the food having a p:c ratio of 1:2 (the p:c ratio ants self-selected in the choice experiment), although mortality was not significantly affected by food p:c ratio. The self-selected p:c ratio in our study is consistent with that observed in other recent ant nutrient regulation studies. Taken together, the results from these combined studies reveal emerging commonalities among ants in macronutrient regulation strategies, and similarities in foraging behaviors and costs associated with macronutrient regulation. Finally, from a methodological perspective, the high mortality observed in our study, when compared with other recent studies, suggests that ant nutrient regulation studies should be conducted using foods having high moisture and total macronutrient content.

Keywords Foraging behavior · Nutrient regulation · Diet selection · Invasive species

Introduction

All organisms must acquire nutrients in appropriate quantities and in the proper ratios to promote fitness. However, 'perfect foods' that meet all these requirements are rarely available in nature. Thus, to meet nutritional requirements, organisms must select among multiple food sources that differ in nutrient content and ratios of key nutrients [e.g., protein (p) and carbohydrate (c)], or when mixing is not possible, feed in such a manner that they most closely match their nutritional requirements. How a remarkable range of organisms do this has been the focus of recent studies, many of which employed the experimental approach of the 'geometric framework'(Behmer, 2009). This approach, first conceptualized by David Raubenheimer and Steve Simpson (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993), explores how animals balance multiple and changing nutrient needs in nutritionally variable environments. Two types of experiments are often conducted when using the geometric approach: choice and no-choice experiments. In choice experiments, organisms are presented with pairs of foods that are complimentary in key nutrients and then allowed to self-select their preferred nutrient intake (both amounts and ratios). This self-selected nutrient 'intake

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target' (sensu Simpson and Raubenheimer, 1993) is functionally significant, as it consistently results in optimal growth and performance (e.g. Behmer and Joern, 2008). In no-choice experiments, organisms are presented with individual foods that have a range of fixed p:c ratios. These no-choice experiments can reveal both how deviations away from a self-selected (optimal) p:c ratio affects performance and foraging behaviors, and modifies the priorities an organism places on regulating nutrients present in excess of requirements, versus nutrients that are in deficit.

Recently the geometric framework has been used to explore how ant colonies regulate nutrient intake (Dussutour and Simpson, 2008, 2009; Cook et al., 2010; Cook and Behmer, 2010). How social insect colonies regulate nutrient intake is of particular interest because, unlike solitary animals, colonies are made up of overlapping generations of individuals that have distinct nutritional requirements, and food is collected by a subset of adult colony members whose nutritional requirements often differ from many of those for which the collected food is intended (Hölldobler and Wilson, 1990; Cassill and Tschinkel, 1999; Cassill, 2003). Despite nutritional differences between caste members, choice experiments reveal that several ant species tightly regulate their protein and carbohydrate intake, at the colony-level, to a specific p:c ratio. In some of these studies, no-choice experiments have demonstrated how colonies meet these requirements through changes in worker foraging behaviors, and through a communal system where select nutrients are extracted from collected foods (Dussutour and Simpson, 2009; Cook et al., 2010). These studies also show elevated colony-level costs associated with efforts to achieve nutritional requirements. For example, ants on protein-rich, carbohydrate-poor foods meet their carbohydrate requirements by collecting large quantities of this food, but then process it to extract all the carbohydrate while dumping large amounts of protein. However, these colonies experienced significantly elevated worker mortality (Cook et al., 2010; Dussutour and Simpson, 2009), possibly as a result of 'food stress' (Chown and Gaston, 1999) induced by handling and processing large amounts of protein.

In the current study, we conducted both dietary choice and no-choice laboratory experiments to explore protein and carbohydrate regulation in the ant, *Nylanderia* sp. nr. *pubens* Forel (Hymenoptera: Formicidae). We quantified total food and protein and carbohydrate collection, and we also quantified worker mortality as a measure of colony-level costs associated with regulating nutrient intake when feeding on nutritionally imbalanced foods. Until recently, *N.* sp. nr. *pubens* was placed in the genus *Paratrechina*. However, a recent revision of the *Prenolepis* genus-group of ants (LaPolla et al., 2010) has reclassified this species as belonging to the genus

Nylanderia. *Nylanderia* sp. nr. *pubens* has been identified as a newly invasive ant species inhabiting portions of the southern United States. The invasive status of this species was first suggested in 2002 after multiple reports of infestations near Houston, TX (Meyers and Gold, 2008). Since that time, this species has spread to nearby states (MacGown and Layton, 2010). A better understanding of this species' nutrient preferences and nutrient regulation behavior may be helpful with designing more efficacious baits. In the discussion, we relate the results of our study to those from similar studies of other ant taxa and make suggestions for further studies.

Materials and methods

Collection of field colonies

All colonies of *Nylanderia* sp. nr. *pubens* used to conduct this study were collected in Pearland, TX (GPS coordinates: N 29°33.518, W 095° 20.531). Colonies used in our experiments were collected monthly (December, 2006–May, 2007). Colonies were located by examining known nesting sites, including under fallen tree limbs, in leaf litter, or in shallow underground nests. Once colonies were located, nesting substrate and ants were shoveled into a bucket (22 L) with its sides treated with talcum powder to prevent the ants from escaping. Colonies were then transported to the Center for Urban and Structural Entomology, Texas A&M University, College Station, TX. The colonies were removed from the soil/debris in the bucket by a drip-floatation method (Banks et al., 1981). One (or more) standard-sized Petri dish(es) (diameter = 8.5 cm, height = 1.5 cm) filled half-way with plaster of Paris was placed on top of the pile of nesting substrate. With the increasing water level in the bucket, ants (some carrying brood) would move up the mound of dirt and into the Petri dish. Once the colony was inside the Petri dish they were removed and placed into a plastic box (30.5 × 16.5 × 8.9 cm) having the inside walls treated with Fluon® (Polytetrafluoro-ethylene, ICI Fluoropolymers INC, Exton, PN, USA) to prevent the ants from escaping. Ants were provided with an artificial nest (Petri dish as described above) and a water/moisture source (75 mL glass jar with a plastic top and cotton wick) and were given ad libitum access to a carbohydrate source (cotton balls soaked in a 20% v/v honey water solution), and a protein/lipid source (crickets). The water, sucrose, and crickets were checked daily and replenished when needed. The colonies were maintained in a growth chamber (Elliott-Williams model: Conviron 8601) at 30 ± 2°C, 12 h:12 h light:dark, and 60% relative humidity (RH) for 3–6 days before creating experimental ant colonies.

Experimental ant colonies and foraging arenas

Each experimental ant colony consisted of one functional queen, 250 workers, and approximately 10 mg of brood (larvae and pupae). Each colony was housed in a glass test tube (1.6 × 15 cm) filled half way with water and plugged with cotton, which acted as both a nest and water source. Experimental arenas consisted of two separate plastic boxes (9 cm high × 16.5 × 30.5 cm) set adjacent to each other. Both boxes had their inside walls treated with Fluon® in order to prevent the ants from escaping. One arena contained the ant colony (nest arena), and the other (foraging arena) contained the experimental foods (described below). The two arenas were connected to each other via a paper bridge (2.5 cm × 45.7 cm) and placed in a constant temperature room maintained at 30–33°C, with RH of 60%, and a 12 h:12 h light:dark photoregime.

Experimental foods

The experimental foods used in this study were dry, granular, and chemically defined, and were prepared as described in Behmer et al. (2001); they were presented to experimental ants as a dry, granular powder. Five foods had an equal total combined amount of protein and carbohydrate content (42%); a sixth food had a smaller combined protein and carbohydrate content (14%). All foods contained identical absolute amounts of other dietary components (e.g., sterols, vitamins); indigestible cellulose powder made up the remaining portion of the diets (Table 1). The six diets used in this study were p7:c35 (7% protein, 35% carbohydrate; expressed as a percent of the total dry mass of the food), p14:c28, p21:c21, p28:c14, p35:c7, and p7:c7.

Experiment 1: Choice

Each month (Dec–May) an experimental colony was allocated to one of two food-pairing treatments—food-pairing 1 (p7:c35 paired with p35:c7) and food-pairing 2 (p7:c35 paired with p28:c14). The experiment was repeated each month (Dec–May), except food-pairing 2, which was included in experiments for Feb-May only (i.e., $N = 6$ and $N = 4$, respectively).

Experimental protocol

Approximately one gram of each food was allocated to a plastic food dish (1 cm high × 2.5 × 2.5 cm) and placed under a 60-watt heat lamp until it had reached a constant mass (to 0.1 mg); the foods sat approximately 30 cm under the lamp, and the temperature where the foods were located varied between 35–38°C. Pre-weighed dried foods were then placed in the foraging arena. Colonies were allowed to

Table 1 Dietary components and amounts of each added to experimental foods used in this study (after Behmer et al. 2001)

Food	Cellulose (g)	Casein (g)	Peptone (g)	Albumen (g)	Sucrose (g)	Dextrin (g)	Linoleic acid (mL)	Cholesterol (mg)	Chloroform (mL)	Wesson's salt (g)	Ascorbate (mg)	Vitamin mix (mg)	20% ethanol (mL)
p7:c7	164.0	8.4	2.8	2.8	7.0	7.0	1.1	0.1	80.0	5.0	0.6	0.4	250.0
p7:c35	108.0	8.4	2.8	2.8	35.0	35.0	1.1	0.1	80.0	5.0	0.6	0.4	250.0
p14:c28	108.0	16.8	5.6	5.6	28.0	28.0	1.1	0.1	80.0	5.0	0.6	0.4	250.0
p21:c21	108.0	25.2	8.4	8.4	21.0	21.0	1.1	0.1	80.0	5.0	0.6	0.4	250.0
p28:c14	108.0	33.6	11.2	11.2	14.0	14.0	1.1	0.1	80.0	5.0	0.6	0.4	250.0
p35:c7	108.0	42.0	14.0	14.0	7.0	7.0	1.1	0.1	80.0	5.0	0.6	0.4	250.0

collect food for 7 days, after which each food dish was removed and replaced with a dish containing a pre-weighed quantity of the same food. Colonies were then allowed to collect food for an additional 7 days. Any food remaining after day 7 and 15 were removed, dried to a constant mass (as described above) and reweighed. The amount of food collected by colonies was determined gravimetrically by subtracting the final dry weight of foods from their initial dry weight (note: colonies never ran out of food during either week of the experiment). Each day, the number of dead workers was counted, and any dead workers were removed.

Experiment 2: No-choice

Each month (Dec–May) an experimental colony was allocated to one of six experimental food treatments (p7:c7, p7:c35, p14:c28, p21:c21, p28:c14, and p35:c7) (i.e., $N = 6$ for each food). The protocol for these experiments was identical to that for choice experiments, except that only one dish of food is provided in no-choice experiments. As in choice experiments, colonies from no-choice experiments never ran out of food during both the first and second week of the experiment.

Statistical analyses

Prior to analyses, data were examined to determine whether data were normally distribution and whether data had equal variances across treatments. If these criteria were not met, data were transformed and parametric statistics performed on transformed data. In choice experiments, because selection by ants was independent between each of the two foods, total food collection and protein and carbohydrate collection were analyzed using a multivariate analysis of variance (MANOVA). In no-choice experiments, food collection, and protein and carbohydrate collection were analyzed with an analysis of variance (ANOVA). Food collection for both choice and no-choice experiments was analyzed for each of the 2 weeks separately and also for the weeks combined. Mortality was analyzed with repeated measures ANOVA. *Post hoc* contrast tests (with alpha adjusted for multiple comparisons) were used to examine across-treatment differences in total food collection, nutrient collection, and worker mortality.

Results

Experiment 1: Choice

The mean (±SEM) total amount of each food collected from each food pairing is shown in Fig. 1a. Across treatments
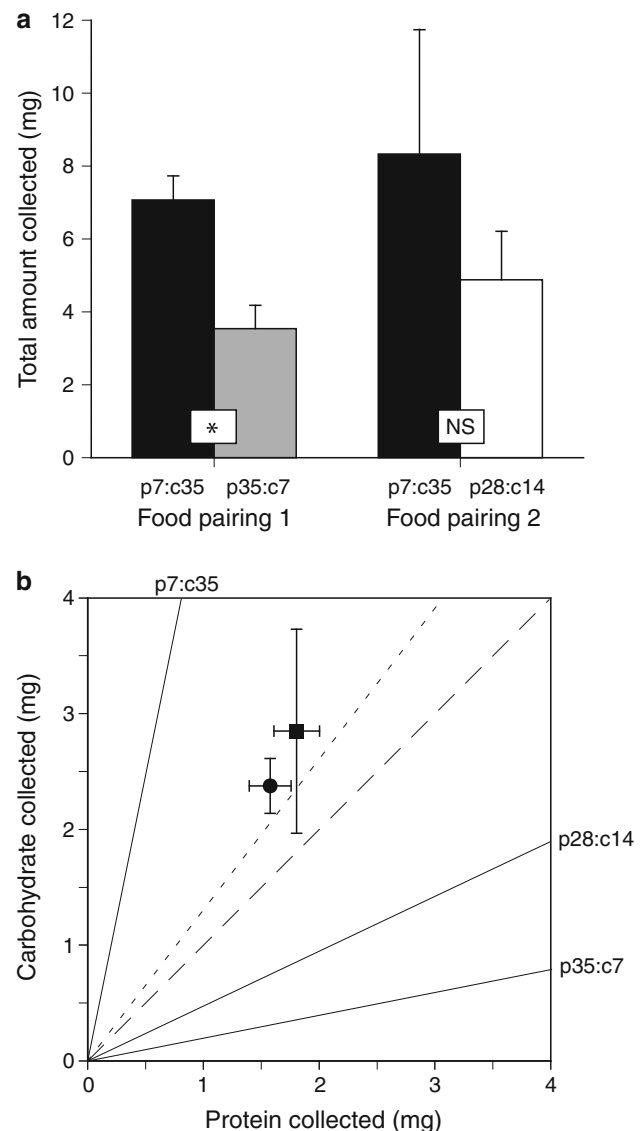
Fig. 1 The mean (±SEM) total amount collected of each food comprising food-pairings in choice experiments (**a**), and the mean (±SEM) amounts of protein and carbohydrate contained in collected foods (**b**). In **a** the '*' and '*NS*' indicate significant and non-significant differences in the respective amounts collected of each of the two foods comprising food pairings, respectively. In **b**, *circle* and *square* symbols represent the protein and carbohydrate intake target of colonies feeding on food paring one and two, respectively. *Solid lines* emanating from the origin represent the protein:carbohydrate ratio (p:c) of experimental foods. *Long* and *short dashed lines* represent the average p:c ratio of food pairings one and two, respectively

most (85%) of the total amount of food collected by colonies was amassed during days 0–7, but the total amount of food collected by ants on the two food pairings was not significantly different over the first 7 days (MANOVA of log-transformed data: $F_{2,7} = 0.19$, $p = 0.828$), for days 8–15 ($F_{2,7} = 1.05$, $p = 0.398$), or for time periods combined ($F_{2,7} = 0.45$, $p = 0.654$). Colonies on food-pairing 1 did not collect from the two food dishes equally (*t*-test:

Table 2 Results from univariate and repeated measures ANOVA ($p < 0.05$) of worker mortality data from no-choice experiment

Experiment		Source	F	df	p
Choice	In-between factors	Food	0.88	1, 10	0.684
	Within factors	Time	59.06	13, 130	<0.001
		Time-by-food	1.39	13, 130	0.576
No-choice	In-between factors	Food	1.28	5, 29	0.299
	Within factors	Time	69.37	13, 377	<0.001
		Time-by-food	1.67	65, 377	0.251

$t_5 = 4.59$, $p = 0.003$), preferring the p7:c35 food to the p35:c7 food. In contrast, colonies on food-pairing 2 collected similar amounts of food from the two available food dishes ($t_3 = 1.46$, $p = 0.121$).

The mean (±SEM) total collected amounts of protein and carbohydrate were not significantly different for each time period (MANOVA of log-transformed data: days 1–7: $F_{2,7} = 0.47$, $p = 0.643$; days 8–15: $F_{2,7} = 0.50$, $p = 0.624$), or for the combined time periods (days 1–15: $F_{2,7} = 0.61$, $p = 0.571$; Fig. 1b). The p:c intake target for colonies of *N.* sp. nr. *pubens* has a p:c ratio close to 1:2. Colonies on the two food-pairings strongly regulated protein intake, although carbohydrate intake was more variable (Fig. 1b).

The mean cumulative worker mortality for colonies on choice food-pairings was similar between the two food-pairings, and colonies from both food-pairings had similar significantly elevated daily mean worker mortality over time (Table 2).

Experiment 2: No-choice

Generally, colonies collected more of the carbohydrate-biased foods (i.e. p7:c35 and p14:c28) relative to the protein-biased foods (p28:c14, p35:c7). Colonies collected relatively small quantities of both the equal-ratio foods (p7:c7, and p21:c21) (Fig. 2a). However, the mean total amount of each of the foods collected was not significantly different across diets for each time period (ANOVA of log-transformed data: days 1–7: $F_{5,30} = 0.17$, $p = 0.688$; days 8–15: $F_{5,30} = 1.03$, $= 0.419$), or for time periods combined ($F_{5,30} = 1.63$, $p = 0.187$, Fig. 2a).

The mean total amount of protein and carbohydrate collected was significantly different across diets for each time period separately (log-transformed data: days 1–7: $F_{5,30} = 3.52$, $p = 0.013$; days 8–15: $F_{5,30} = 4.57$, $p = 0.003$) and for time periods combined (days 1–15: $F_{5,30} = 6.22$, $p < 0.001$; Fig. 2b).

Among no-choice foods, colonies feeding on p14:c28 diet had the fewest mean cumulative number of dead ants. However, mean cumulative worker mortality was similar across the no-choice treatments (Fig. 3a). Mean daily

mortality increased significantly over time for colonies feeding on all no-choice foods (Table 2).

Finally, we compared food collection and performance data from choice and no-choice experiments. Colonies from choice experiments collected significantly greater quantities of total food (ANOVA of cube root-transformed data; $F_{1,44} = 9.01$, $p = 0.004$). A comparison of worker mortality between choice and no-choice experiments revealed that mean cumulative worker mortality was not significantly different (ANOVA of log-transformed data: $F_{1,45} = 2.91$, $p = 0.091$), but colonies from choice experiments had significantly lower mean daily worker mortality during the first half of the experiment (repeated measures ANOVA of log-transformed data, time-by-treatment interaction: $F_{13,585}$, $p = 0.044$, Fig. 3c).

Discussion

The strength of the geometric framework is that it lets an organism reveal its preferred nutrient 'intake target' (particularly the preferred ratio of key nutrients). Our results reveal that despite *N.* sp. nr. *pubens* workers showing a strong preference for carbohydrate-rich foods, colonies self-selected a diet having only a moderately carbohydrate-biased p:c ratio ($\sim$1:2). Interestingly, this self-selected p:c ratio is similar to those obtained for three other ant taxa, including *Rytidoponera metallica* (Dussutour and Simpson, 2009), *Ectatomma ruidum* (Cook and Behmer, 2010), and *Solenopsis invicta* (Cook et al., in review). Two of these species, *R. metallica* and *E. ruidum*, are closely related to one another (subfamily Ectatomminae (Moreau et al., 2006), but all three are considered predatory ants based on nitrogen isotopic analyses of body tissues (Davidson et al., 2003). *N.* sp. nr. *pubens* was, until recently, placed in the genus *Paratrechina* (LaPolla et al., 2010) (above), and within the Formicinae, *Paratrechina* species appear to be relatively predatory compared with other more 'herbivorous' members of that subfamily (Davidson et al., 2003). However, one conspicuous difference between *N.* sp. nr. *pubens* and the three other ant species that have been studied using the geometric framework is the erratic and rapid foraging tempo of *N.* sp. nr. *pubens* workers (hence the term 'crazy ant'). Oster and Wilson (1978) originally distinguished between 'low' and 'high' tempo ants, and high tempo species are predicted to show a preference for strongly biased carbohydrate diets to fund seemingly energetically wasteful foraging activity and/or collect dietary carbohydrate in excess of amounts necessary for colony growth and reproduction (Davidson, 1997). That colonies of *N.* sp. nr. *pubens* in the current study self-selected diets having a p:c ratio very similar to more slow-tempo species suggests that there may be factors other than access to carbohydrate that distinguish 'high-tempo' from
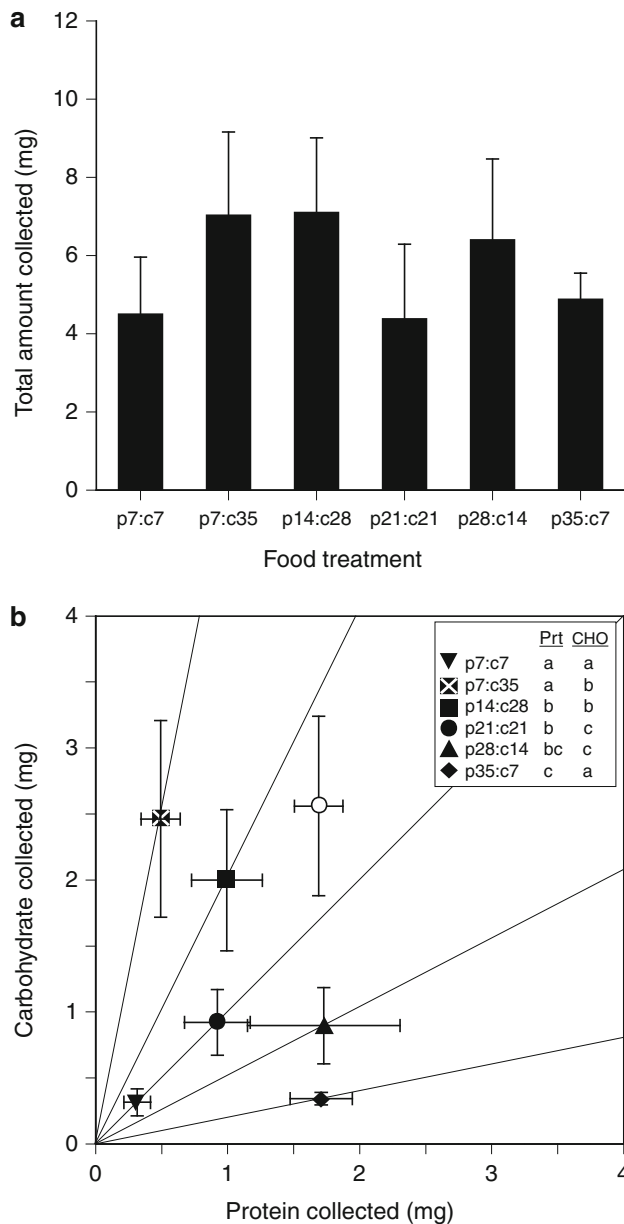
Fig. 2 The mean (±SEM) total amount collected by colonies of each no-choice food (**a**), and the mean (±SEM) amounts of protein and carbohydrate contained in collected foods (**b**). In **b**, *symbols* represent each of the no-choice foods, and *lines* emanating from the origin represent the p:c ratio of those foods. *Lower case letters* in the figure legend show statistical differences (from selective contrast post-hoc tests with alpha adjusted for multiple comparisons) in the amount of protein and carbohydrate collected by colonies feeding on no-choice foods. The *open circle* is the average amount of protein and carbohydrate eaten in the choice experiment (see Fig. 1b)

'low-tempo' species and 'herbivorous' from 'predatory' species.

The use of no-choice experiments, employing a range of foods that differ in their p:c ratios, allows researchers to measure the response of an organism to nutrient limitation, and can reveal the priorities an organism places on
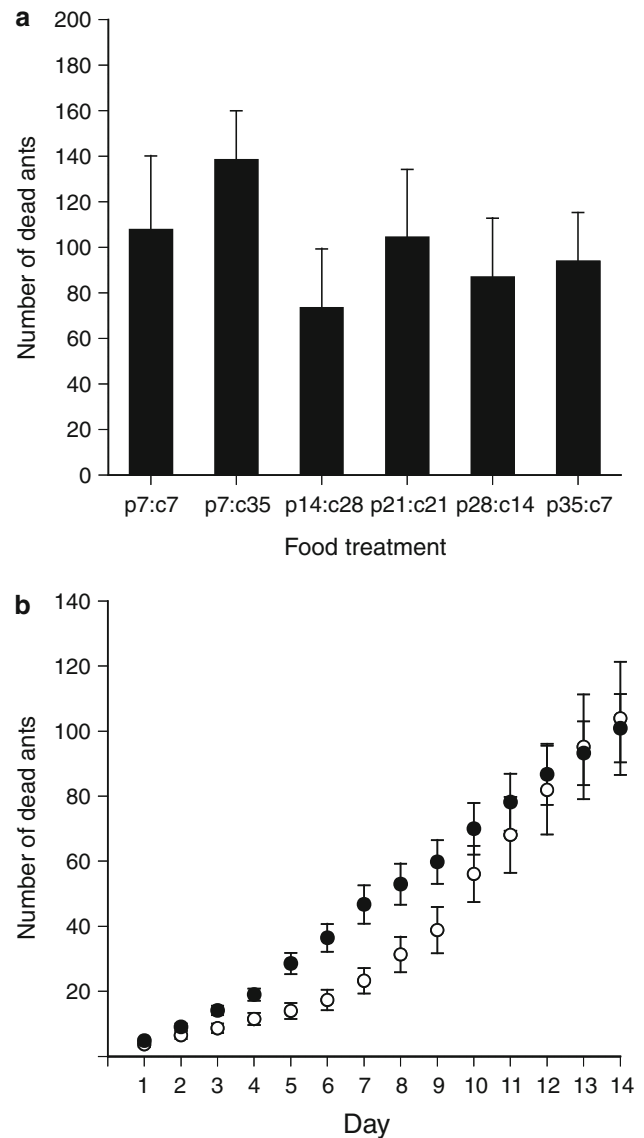
Fig. 3 The mean (±SEM) total cumulative number of dead workers from colonies feeding on no-choice foods **a**, and **b** the daily mortality of colonies from choice (*open symbols*) and no-choice (*closed symbols*) experiments

simultaneously regulating the intake of multiple nutrients. If ant colonies feeding on a single, nutrient-imbalanced food regulate intake of the limiting nutrient to meet requirements for this nutrient, colonies should increase the amounts of food they collect as available foods become increasingly nutritionally imbalanced (relative to that species' needs). In our study, *N.* sp. nr. *pubens* colonies showed only a moderate response to the nutrient content of experimental foods and collected only slightly more of the foods deficient in either nutrient. Results from the no-choice experiments suggest that when colonies of this species are challenged to meet nutrient requirements from a single food source, they collect enough of the nutrient-imbalanced foods to meet

requirements of the non-limiting nutrient. In contrast, *Solenopsis invicta* colonies show the predicted pattern in food collection indicative of active regulation of the limiting nutrient (Cook et al., in review). It is possible that had our experiments run for a slightly longer amount of time, and used larger initial colony size, *N*. sp. nr. *pubens* colonies may have regulated for the limiting nutrient. Additionally, the dilute nature of the experimental foods (total macronutrient content of 42%, and in one extreme case 14%) may have been too great for colonies to regulate intake of the limiting nutrient via compensatory feeding (but see Cook et al., (2010), and below). Instead, they may rely on physiological mechanisms (e.g., efficient manipulation of collected food, differential utilization of ingested nutrients) to compensate for decreased collection of limiting nutrients.

Plotting the protein and carbohydrate content of amassed no-choice foods reveals that colonies feeding on moderately nutrient-imbalanced foods successfully met their intake target for only the non-limiting nutrient. However, colonies feeding on the most nutritionally dilute food (p7:c7) did not meet intake requirements for either protein or carbohydrate. These combined results suggest that *N*. sp. nr. *pubens* colonies used in our experiments do not possess strong compensatory capabilities, especially with respect to collecting large amounts of food to make up for deficits in limiting nutrients. In contrast, experimental colonies of *Solenopsis invicta* will collect excess non-limiting nutrients to more closely match nutrient requirements (Cook et al., in review). A similar response was observed for *Rytidoponera metallica* colonies feeding on dilute carbohydrate solutions (Dussutour and Simpson, 2009), but in this case, large volumes of water were collected. Colonies feeding on our p7:c7 diets amassed only enough food to reach the protein or carbohydrate intake of colonies feeding on the most protein- and carbohydrate-biased foods. Interestingly, colonies did collect this latter food in similar amounts to colonies on other foods, but suffered nutrient deficits. Perhaps colonies collected enough food to satisfy a requirement for a particular quantity of food, but did not collect excess dilute food to avoid possible 'food stress'. Alternatively, the low macronutrient content of our experimental food (as compared with an animal prey item, which would have high absolute amounts of protein and lipid) may have deterred workers from collecting it in large quantities.

Sometimes there are costs associated with nutrient regulation. For example, worker mortality is often high on nutritionally imbalanced foods, particularly those that are protein-biased (Dussutour and Simpson, 2009; Cook et al., 2010; Cook et al., in review). In our study worker mortality was very high (about 40% over 2 weeks), and no significant statistical differences in mortality were observed across our eight treatments. Nevertheless, three observations suggest that a diet having a p:c ratio of approximately 1:2 is likely optimal for *N*. sp. nr. *pubens*. First, mean total mortality was lowest (albeit not significantly) in colonies feeding on food p14:c28. Second, mean daily worker mortality during the first half of the experiment was significantly lower on the choice treatments. Third, food collection was greatest when ants were allowed to self-select their diet. However, two factors likely masked any benefit of eating a diet with a 1:2 p:c ratio. One, the nutritionally dilute nature of the foods— 42% total macronutrient content (and 14% in one extreme instance), compared with ∼80% in other studies (Cook et al., 2010; Cook et al., in review) reduced the total amount of macronutrients colonies could collect. Two, the dry nature of our test food may have imposed significant physiological costs because formicine ants (including *Nylanderia* spp., or at least their ancestors) are generally "built" for feeding on liquid foods. Despite any negative impact of these last two factors on survival, the general results we have obtained in the current study, with respect to nutrient regulation in choice and no-choice experiments, are in line with observations reported for other ant species.

Our study adds to the growing number of those using the geometric framework to study the nutritional biology of social insects. These collective studies are beginning to reveal across-species commonalities in the protein and carbohydrate content of self-selected diets and document contrasting nutrient regulation strategies. Future studies should focus on species that differ greatly in observed nutrient niches (Davidson et al., 2003; Davidson, 2005; Behmer and Joern, 2008) to determine whether these ants differ in basic nutritional needs. If species are shown to self-select diets similar in the ratio of key nutrients, it would suggest that ant species diversity might be promoted through contrasting physiologies (high tempo versus low tempo), and occupation of vastly different ecological niches (e.g. terrestrial versus arboreal). An additional take home message from the current study, compared with other recent studies (e.g. Dussutour and Simpson, 2009; Cook et al., in review), is that ants perform better on agar-based diets that have high total macronutrient content.

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