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# Nutrient stores predict task behaviors in diverse ant species

R. E. Silberman<sup>1,2</sup> · D. Gordon<sup>1,3</sup> · K. K. Ingram<sup>1</sup>

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**Abstract** In eusocial species, including ants and honeybees, sterile or non-reproductive workers can specialize in task-specific behaviors, such as brood care and foraging for food. The mechanisms underlying task-specific behaviors include genetic, physiological and environmental factors. Here we compare corporeal nutrient storage in nine species that differ in primary food preferences (carbohydrate-, protein- or lipid-based diet) to test whether foraging behavior is associated with lower individual nutrient stores. We also investigate whether low nutrient stores are limited to foragers or occur in other external, morphologically distinct, worker sub-castes. In six out of eight species where both brood care workers and foragers were sampled, foragers had significantly lower nutrient stores relative to brood care workers; the exceptions were two *Solenopsis* species. Foragers from five of these six species had lower lipid levels, supporting the link between lipid content and foraging behaviors reported in previous studies. Interestingly, three species had lower levels of both lipid and carbohydrate stores in foragers relative to brood care workers, and foragers of one species, *Formica fusca*, had lower carbohydrate levels but not lipid levels, suggesting that the association between nutrient stores and foraging behavior is not universal across ant species or across all

seasons. In all three species with morphologically distinct sub-castes, lipid levels were lowest in non-foraging, external workers, i.e., majors or soldiers, indicating an additional link between nutrient depletion and the allocation of external tasks other than foraging.

**Keywords** Task allocation · Division of labor · Social insects · Foraging · Nutrient stores

## Introduction

In evolutionarily derived eusocial lineages of ants, species exhibit both a reproductive division of labor and a behavioral division of labor where sterile or non-reproductive workers perform specialized task-specific behaviors, such as brood care or foraging (Toth and Robinson 2007; Smith et al. 2008). Efficient task allocation is critical for the survival and fitness of the colony (Wilson 1975). Task allocation can occur as an age-related polytheism where younger non-reproductive individuals remain in the nest to care for the young before transitioning to out-of-nest foraging behaviors. Alternatively, task allocation could be due an expansion in the task repertoire with ants acquiring new tasks as they develop and external foraging behavior appearing later in development (Seid and Traniello 2006). In some species, workers can develop into morphologically distinct sub-castes that do not switch tasks as they age. For example, the ant, *Atta cephalotes*, maintains behaviorally distinct sub-castes that range widely in size, physical appearance, and behavior (Wilson 1983).

Foraging behaviors, including locating and transporting resources back to the nest, are tasks common to most ant species with a behavioral division of labor. In species with age-related polyethism, foraging tasks tends to be

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✉ K. K. Ingram  
kingram@colgate.edu

<sup>1</sup> Colgate University, 13 Oak Drive, Hamilton, NY 13346, USA

<sup>2</sup> Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA

<sup>3</sup> Boston University, One Silber Way, Boston, MA 02215, USA

performed by older workers; in species with morphologically distinct sub-castes, foragers specialize in this behavior and rarely perform other behaviors such as brood care or nest maintenance. Advances in understanding the physiological mechanisms underlying the onset of foraging behavior have focused on differences in juvenile hormone (Robinson 1987; Robinson et al. 1989, reviewed in Robinson 1992), factors such as ovarian or fat body development (Kondoh 1968; Porter and Jorgensen 1981; MacKay 1983; Hölldobler and Wilson 1990; O'Donnell and Jeanne 1995; Fénéron et al. 1996) and on individual and colony nutrient stores (Wheeler and Martinez 1995; Blanchard et al. 2000; Toth et al. 2005; Toth and Robinson 2005; Hahn 2006; Daugherty et al. 2011; Smith et al. 2011; Mayack and Naug 2013). In this paper, we focus on the links between foraging behavior, food preference and worker nutrient levels in multiple ant species.

Individual differences in nutrient storage have been linked to the behavior of both solitary (Pereira and Sokolowski 1992; Kaun et al. 2008) and social insects (Tschinkel 1998; Toth and Robinson 2005; Toth et al. 2005). In honeybees, foragers have lower lipid stores than nurse bees, independent of age or foraging experience; low lipid levels appear to precede the onset of foraging (Toth and Robinson 2005). Young honeybees treated with 5-(*tetradecyloxy*)-2-furoic acid (TOFA), an acetyl CoA carboxylase inhibitor that prevents lipid storage, demonstrated precocious foraging (Toth et al. 2005). In the primitively eusocial paper wasp, *Polistes metricus*, food-deprivation and reduced lipid stores were also associated with increased foraging behavior (Daugherty et al. 2011).

All ants have the ability to store nutrients within their crops, fat bodies, and as corporeal protein, including structural, enzymatic, and hemolymph proteins (Stradling 1987) with the most common reserve as internal fat storage (Blanchard et al. 2000; Hahn 2006). Protein and lipid stores differ during ant development (Wheeler and Buck 1992; Wheeler and Martinez 1995; Tschinkel 1998) and at different times of the year (Vinson 1968; Tschinkel 1993; Judd 2006). In some ants, lipid or fat stores have been found to be negatively associated with foraging propensity (Tschinkel 1998; Blanchard et al. 2000; Hahn 2006); these studies have involved species that utilize a primarily lipid-based energy source. Little is known about ant species that rely on carbohydrate- or protein-based metabolism and whether these species share the negative association between lipid depletion and behavior, or whether reduced stores of alternative energy sources provide foraging cues. In 1979, Martin and Lieb assessed the relative activity of metabolic enzymes in the thoracic muscles of three ant species and found that enzymatic activity reflects the dietary regime of the species. *Pogonomyrmex californicus*, a granivorous ant, had high levels of enzymes involved in lipid breakdown while

*Formica ulkei*, a sugar-based species, emphasized glucose metabolism and *Atta columbicus*, a fungus feeder, utilized enzymes for metabolism of both lipids and carbohydrates (Martin and Lieb 1979). If lipid depletion drives the onset of foraging in social insects, one would expect a negative association between foraging behavior and lipid stores in all ant species, regardless of resource or metabolic preference. Recently, Smith et al. (2011) found evidence for a nutritionally based division of labor in the queenless Ponerine ant, *Dinoponera australis* with foraging workers more likely to have decreased fat reserves. Hahn (2006) found subspecies-specific differences in nutrient storage profiles in the ant species complex, *Camponotus festinates* (Hahn 2006), but these differences have not yet been linked to behavioral differences. Seasonal food preference in *Solenopsis invicta* (Porter et al. 1987) and the relationship of seasonal food preference with individual corporeal nutrient stores in *Pheidole ceres* (Judd 2006) have also been investigated, but generally little is known about the interactions between resource preference, task-specific behavior and individual energetic state in ants.

To explore the species- and task-specific properties of nutrient storage in ants, we quantified individual corporeal carbohydrates and lipids from workers of nine species. In eight of these species, *Pogonomyrmex occidentalis*, *Pogonomyrmex barbatus*, *F. fusca*, *A. cephalotes*, *S. invicta*, *Solenopsis geminata*, *Pheidole spadonia* and *Nylanderia fulva*, we compared the nutrient levels between brood care workers inside the nest and foragers that travel outside the nest. To test if low nutrient stores are specific to foragers or shared with other external workers, we examined three species, *Camponotus floridanus*, *P. spadonia*, and *A. cephalotes*, with morphologically distinct sub-castes. If nutrient depletion is functionally related to foraging behavior rather than environment, distance from nest brood chambers or some other factor, foragers are expected to have lower nutrient stores than both internal brood care workers and externally located, non-foraging workers.

## Methods

### Sample collection

We quantified individual corporeal carbohydrates and lipids from workers of nine species (Table 1) during the early to late spring. In field-collected colonies, foragers were identified as workers carrying food outside the nest. Brood care workers were collected by digging into the nests until brood was seen; workers in brood chamber actively interacting with brood were immediately collected as brood care workers. When collecting workers from lab colonies, foragers were identified as workers carrying or interacting with

**Table 1** Summary of species sampled in study

Species	Preferred food sources	Castes sampled	References
Western harvester ant <i>Pogonomyrmex occidentalis</i>	Seeds [lipid/carbohydrate]	Brood care, forager	Fewell (1988)
Red harvester ant <i>Pogonomyrmex barbatus</i>	Seeds [lipid/carbohydrate]	Brood care, forager	Gordon (1995)
Black wood ant <i>Formica fusca</i>	Sugars, invertebrates [carbohydrate/protein]	Brood care, forager	Wallis (1962)
Red imported fire ant <i>Solenopsis invicta</i>	Generalist [protein/carbohydrate/lipid]	Brood care, forager	Porter et al. (1987), McGlynn (1999)
Tropical fire ant <i>Solenopsis geminata</i>	Generalist [protein/carb/lipid]	Brood care, forager	Risch and Carroll (1982), McGlynn (1999)
Tawny crazy ant <i>Nylanderia fulva</i>	Sugar, invertebrates [carbohydrate/protein]	Brood care, forager	Zenner-Polanía (1990; Cook et al. 2011)
<b>Species with distinct sub-castes</b>			
Florida carpenter ant <i>Camponotus floridanus</i>	Sugar [carbohydrate]	Major, minor	Morel et al. (1987)
No common name <i>Pheidole spadonia</i>	Invertebrates/sugars [protein/carbohydrate]	Brood care, forager, major	Wheeler and Buck (1992)
Leaf-cutter ant <i>Atta cephalotes</i>	Fungus [lipid/carbohydrate]	Small, medium, large	Wilson (1983)

Common names, preferred food sources, and the castes sampled for the nine sampled species

food in the external foraging arena. Brood care workers were collected from covered, separate brood chambers and were verified to be interacting with brood. Minor and major workers of *C. floridanus*, major workers of *P. spadonia* and small, medium, and large workers of *A. cephalotes* were identified by size. Laboratory colonies were maintained at 25.5 degrees C, 55 % humidity, 12:12 L:D.

*Formica fusca* colonies ( $n = 6$ ) were field-collected in Hamilton, New York. Ants were transferred into laboratory colony boxes at Colgate University, Hamilton, NY, in 12:12 light:dark conditions for less than 24 h. The ants were not provided with a food source. Samples were frozen at  $-80\text{ }^{\circ}\text{C}$  at 8 a.m., after spending the night in the lab. Samples remained at  $-80\text{ }^{\circ}\text{C}$  until analysis.

Partial *P. barbatus* colonies (workers and brood without queen,  $n = 4$ ) were field-collected in Arizona, immediately frozen, and shipped at  $-70\text{ }^{\circ}\text{C}$  to Colgate University. Partial *P. occidentalis* colonies (workers and brood without queen,  $n = 4$ ) were field-collected in Utah and shipped alive to Colgate University where they were kept alive in lab colonies for weeks in 12:12 light: dark conditions and fed with 50 % sucrose gel and a commercially supplied mixture of grass seeds, grains and unsalted, hulled sunflower seeds, ad libitum, before freezing at  $-80\text{ }^{\circ}\text{C}$ .

*Pheidole spadonia* colonies ( $n = 3$ ) were field-collected in Tuscon, Arizona before being housed in colonies at

Boston University in Massachusetts for months. *Camponotus floridanus* colonies ( $n = 5$ ) were field-collected in Gainesville, Florida before being housed in colonies at Boston University for months. Both species of ants were fed 1 M sucrose and live insect prey (meal worms or fruit flies) or scrambled eggs every other day. *Atta cephalotes* colonies ( $n = 3$ ) were field-collected in Trinidad before being housed in colonies at Boston University for months. Ants were given organic kale/spring mix/orange peel ad libitum for use in cultivating fungus gardens. Individuals were flash frozen using liquid nitrogen and shipped frozen to Colgate University for analysis.

*Solenopsis invicta* and *S. geminata* colonies ( $n = 3$  colonies per species) were maintained at the USDA in Gainesville, Florida for months and fed crickets (frozen) and 10 % sugar water and water ad libitum. *Nylanderia fulva* colonies ( $n = 3$ ) were maintained at the USDA in Gainesville, Florida for months and fed stable fly larvae, crickets (frozen), 10 % sugar water and water ad libitum. Individuals were flash frozen using liquid nitrogen and shipped frozen to Colgate University for analysis.

### Sample preparation

For morphologically larger species (*P. barbatus*, *P. occidentalis*, *F. fusca*, *P. spadonia* majors, and large *A. cephalotes* workers) the bodies of three ants of the same colony and

worker task/sub-caste were pooled and represent one sample per task per colony. For remaining species, a larger number of individual ants of the same nest and worker task/sub-caste were pooled and homogenized together in a single tube to produce samples of similar mass as those in morphologically larger species. Exact masses were recorded for per-mass measurements of lipid and carbohydrate composition.

### Carbohydrate analysis

Samples were weighed and then homogenized in 160  $\mu\text{L}$  distilled water using QIAshredder columns (Qiagen). Two 70  $\mu\text{L}$  extracts were taken and processed separately. The average of these two points was taken as a sample data point. Replicate data points per task were collected across colonies ( $n = 3\text{--}6$  colonies, depending on species). The carbohydrate extraction procedure was modified from the protocol described in Judd (2006); extractions were quantified using dextrose standards in water. Ant extracts were treated with 3 mL anthrone (100 mmol) in conc. sulfuric acid, and heated at 100  $^{\circ}\text{C}$  for 12 min. Absorbance was read at 625 nm on a Beckman Coulter spectrophotometer. Carbohydrate contents were found through interpolation to a standard series (range of  $R^2$  for standards = 0.93–0.99). Differences between worker groups were analyzed using one-tailed  $t$  tests (species with two tasks) or one-way ANOVAs (species with more than two tasks/sub-castes). All statistics were analyzed in SPSS.

### Lipid analysis

Samples were weighed, then homogenized in 160  $\mu\text{L}$  isopropanol using QIAshredder columns (Qiagen). One extract was obtained per task/caste for each colony and species. Replicate data points per task were collected across colonies ( $n = 3\text{--}6$  colonies). Using corn oil standards in isopropanol, total lipids were extracted following a standard procedure (Bligh and Dyer 1959). Extracts were treated with 100  $\mu\text{L}$  potassium hydroxide (1 M) and incubated for 5 min at 60  $^{\circ}\text{C}$ . Samples were treated with 100  $\mu\text{L}$  sodium periodate (1 mmol) in 2 M acetic acid and let to sit at room temperature (25  $^{\circ}\text{C}$ ) for 10 min. 2 mL of color reagent (acetylacetone (0.2 %), isopropanol (66 %), and 2 M ammonium acetate (33 %)) was added. The solution was heated for 30 min at 60  $^{\circ}\text{C}$  and absorbance was read at 410 nm on a Beckman Coulter spectrophotometer. Lipid contents were found through interpolation to a standard series (range of  $R^2$  for standards = 0.94–0.97). Differences between tasks were analyzed using one-tailed  $t$  tests (species with two tasks) or one-way ANOVAs (species with more than two tasks/sub-castes). All statistics were analyzed in SPSS.

## Results

To assess the role of individual nutrient storage in task behavior, carbohydrates and lipids were quantified by spectrophotometric assay in nine ant species. Five out of eight species for which foraging and brood care workers were available had significantly lower lipid levels in foragers. Seven out of nine species had significant differences in nutrient stores across tasks/sub-castes with one species showing a similar trend.

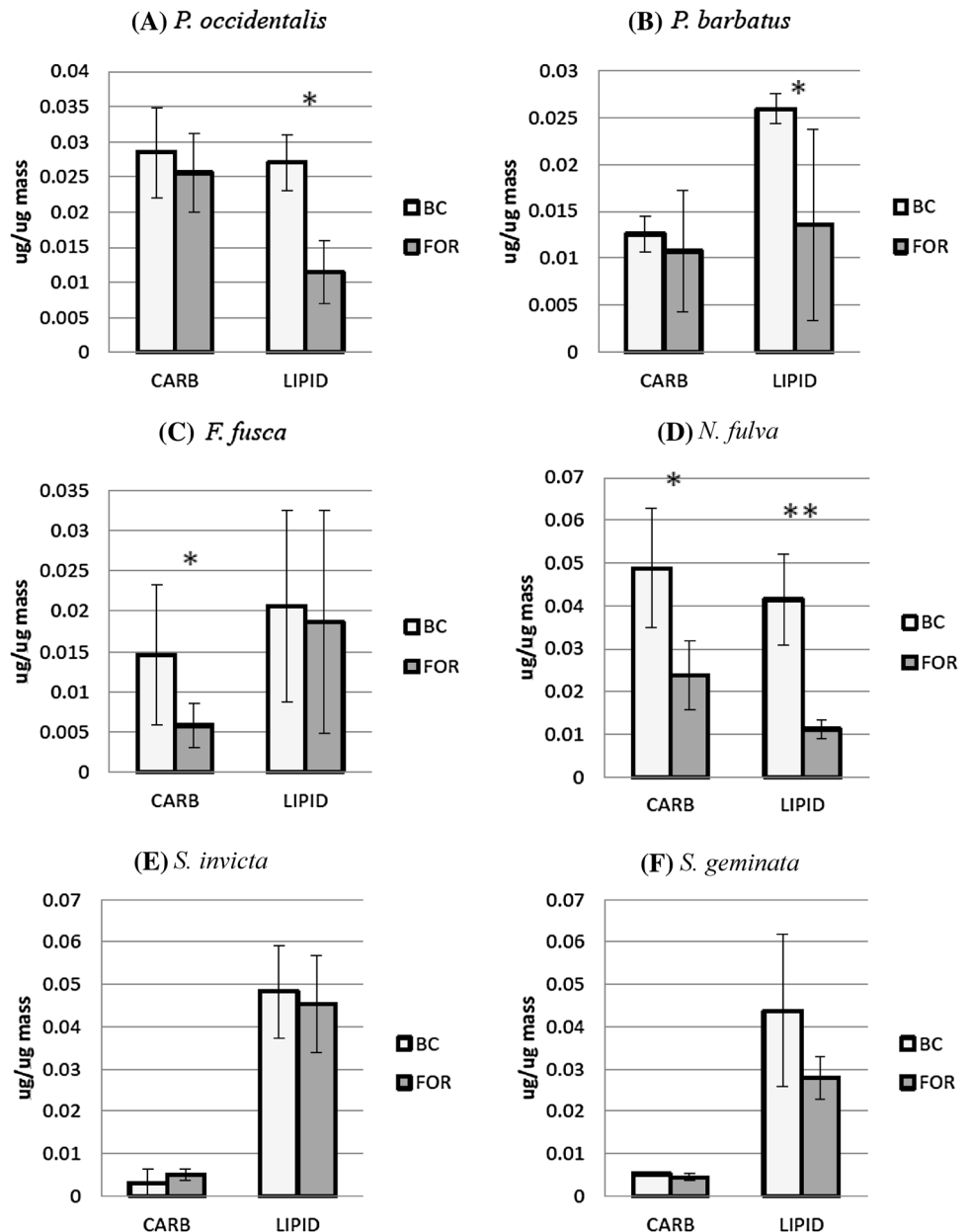
Three species, *P. occidentalis*, *P. barbatus*, and *F. fusca*, showed significant differences between tasks in only one nutrient (Fig. 1a–c). Foragers of the granivorous *P. occidentalis* and *P. barbatus* species had significantly lower lipid stores ( $t = 2.49$ ,  $p = 0.047$ ,  $df = 6$ ;  $t = 2.93$ ,  $p = 0.026$ ,  $df = 4$ ) but not carbohydrate stores ( $t = 1.32$ ,  $p = 0.234$ ,  $df = 6$ ;  $t = 1.12$ ,  $p = 0.305$ ,  $df = 4$ ) compared to brood care workers. In contrast, foragers of *F. fusca* prefer carbohydrate-rich food sources and demonstrated significantly lower carbohydrate stores ( $t = 2.75$ ,  $p = 0.020$ ,  $df = 10$ ) but not lipid stores ( $t = 0.87$ ,  $p = 0.402$ ,  $df = 10$ ).

Three species, *A. cephalotes*, *P. spadonia* and *N. fulva*, showed lower levels of both carbohydrates and lipids in foragers relative to brood care workers (Figs. 1d, 2b, c). *Nylanderia fulva* foragers showed a significant difference in both lipid ( $t = 5.94$ ,  $p = 0.004$ ,  $df = 4$ ) and carbohydrate storage ( $t = 3.37$ ,  $p = 0.030$ ,  $df = 4$ ). In *A. cephalotes*, medium-sized workers act as foragers and the smallest workers perform brood care and internal nest maintenance. *Atta cephalotes* sub-castes varied in both lipid ( $F = 25.52$ ,  $p = 0.002$ ,  $df = 2$ ) and carbohydrates ( $F = 17.91$ ,  $p = 0.005$ ,  $df = 2$ ) with homogeneous subsets indicating a significant difference between workers that engage in foraging versus brood care. *Pheidole spadonia* colonies maintain a morphologically distinct major caste for defense; in a related species, *Pheidole pallidula*, major workers can convert to foraging behavior (Lucas and Sokolowski 2009). A one-way ANOVA indicated significant variance between the three task groups (brood care workers, minor and major workers) for both lipids ( $F = 59.19$ ,  $p < 0.000$ ,  $df = 2$ ) and carbohydrates ( $F = 14.66$ ,  $p = 0.008$ ,  $df = 2$ ), with homogeneous subsets indicating a significant difference between brood care and major workers for carbohydrates and a significant difference between all three groups for lipids.

The invasive Solenopsis species, *S. invicta* and *S. geminata* did not show significant differences in either lipid (Fig. 1e, f;  $t = 0.98$ ,  $p = 0.383$ ,  $df = 4$ ;  $t = 2.08$ ,  $p = 0.106$ ,  $df = 4$ ) or carbohydrate storage ( $t = 1.47$ ,  $p = 0.214$ ,  $df = 4$ ;  $t = 2.07$ ,  $p = 0.108$ ,  $df = 4$ ).

Morphologically distinct sub-castes were studied in three species: *C. floridanus*, *P. spadonia*, and *A. cephalotes*. All

**Fig. 1** Patterns in relative nutrient storage between foragers and brood care workers differ across species. Foragers have lower lipid content than brood care workers in *P. occidentalis* (a) and *P. barbatus* (b), lower levels of carbohydrates in *F. fusca* (c) and lower levels of both lipids and carbohydrates in *N. fulva* (d). No significant difference between task groups were found in either lipid or carbohydrate stores for *S. invicta* (e) or *S. geminata* (f). \* $p < 0.05$ , \*\* $p < 0.005$

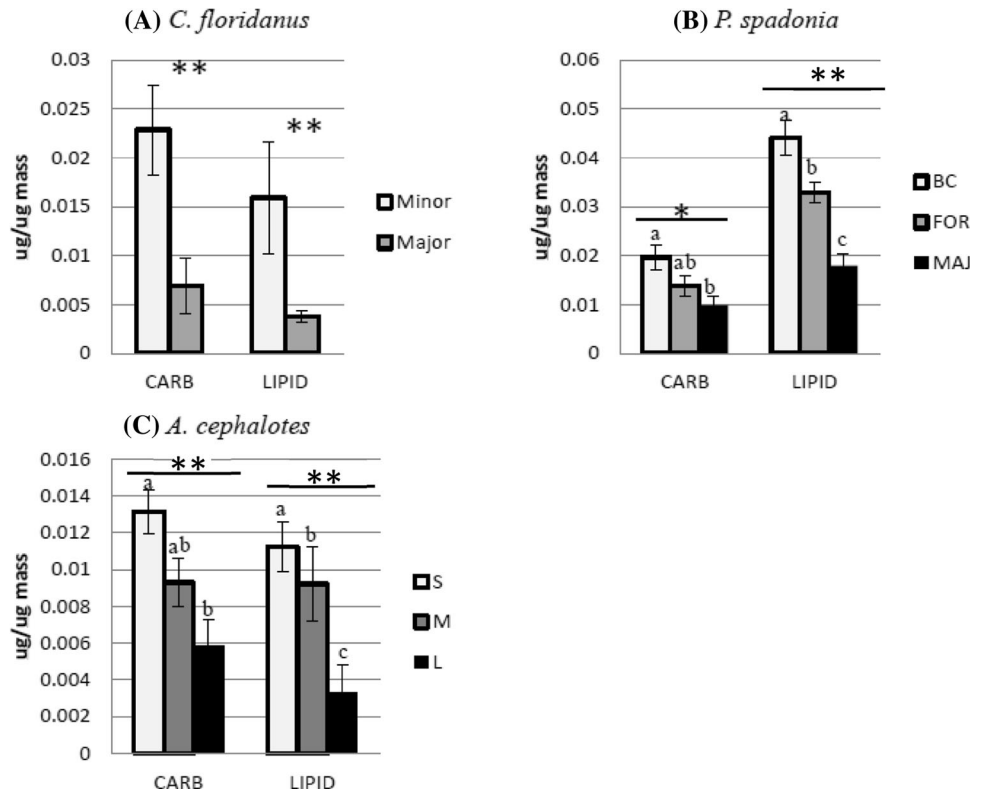


three species showed significant differences between sub-castes in both carbohydrates and lipid content, with non-foraging external sub-castes having the lowest lipid levels (Fig. 2a–c). *Camponotus floridanus* minor workers are associated with foraging while majors are associated with defense (Gronenberg et al. 1996). Major workers demonstrated significantly lower lipid ( $t = 5.29$ ,  $p < 0.000$ ,  $df = 8$ ) and carbohydrate stores ( $t = 7.30$ ,  $p < 0.000$ ,  $df = 8$ ) compared to minor workers. We did not have access to brood care workers in this species. For both *A. cephalotes* and *P. spadonia*, homogeneous subsets indicate that large workers or majors had significantly lower lipid levels than the foraging sub-castes ( $F = 25.52$ ,  $p = 0.002$ ,  $df = 2$  and  $F = 59.19$ ,  $p < 0.000$ ,  $df = 2$ , respectively).

## Discussion

For a majority of species in this study, workers of different tasks and sub-castes vary in nutrient storage (carbohydrate or lipid), regardless of resource specialization or phylogenetic history. Of the eight species for which foragers and brood care workers were available, six species had significantly lower nutrient levels in foraging workers relative to brood care workers and one species showed a similar trend, suggesting that the association between foragers and nutrient depletion reported in previous bee and wasp studies also occurs in ants. The diversity of ants examined in this study represents multiple sub-families, ecotypes, and nutrient resource niches. Across this diversity in life histories and

**Fig. 2** External, non-foraging workers have lower nutrient stores relative to foragers and internal workers. **a** Significantly lower lipid and carbohydrate stores were found in major *C. floridanus* workers relative to minor workers. In addition, significant variation in both lipid and carbohydrate stores were found across worker sub-castes in both *P. spadonia* (**b**) and *A. cephalotes* (**c**). \* $p < 0.05$ , \*\* $p < 0.005$



metabolic preferences, there is a signature of low nutrient stores in foragers relative to brood care workers. However, not all ant species showed identical patterns and two species had no significant differences in nutrient stores between tasks. Furthermore, all three species with morphologically distinct sub-castes showed significant differences between sub-castes in both carbohydrates and lipid content, with non-foraging external sub-castes having the lowest nutrient levels. These results suggest that the link between nutrient stores and task behavior may be more complex than previously thought.

### Lipid depletion and foraging behavior

Of the eight species where brood care workers and foragers were available for analysis, five of these species had significantly lower lipid stores in foraging workers relative to brood care workers and one species, *S. geminata*, shows a similar trend. The data from these species support the hypothesis that lipid depletion is associated with foraging behavior in some ants (Blanchard et al. 2000; Smith et al. 2011) as well as other social insect species (Toth and Robinson 2005; Toth et al. 2005; Daugherty et al. 2011). Interestingly, two species, *S. invicta* and *F. fusca*, did not show the expected pattern in lipid stores, suggesting that the relationship between lipid stores and foraging behavior may not be universal across ant species.

The depletion of individual nutrient stores has been implicated as a molecular mechanism behind task allocation. For example, the inhibition of lipid deposition during worker development induces a precocious transition to foraging behavior in honeybees (Toth et al. 2005). In honeybees, Toth et al. (2005) found that a roughly 20 % lower lipid content was sufficient to induce foraging behavior in young bees. Although a similar experimental study has not yet been done in ants, we calculated the percent difference in carbohydrates and lipids between tasks/sub-castes and compared to the 20 % threshold found in bees (Suppl. Table S1). Results from this study exceeded the 20 % threshold for most species, suggesting that the differences between tasks or sub-castes may be sufficient to impact behavior. The only exception to this pattern occurs in *P. spadonia* between brood care workers and foragers; the percent difference (14.64) is close to the threshold found in honeybees. *S. invicta* and *S. geminata* showed differences greater than 20 % that were not statistically significant.

Interestingly, *F. fusca*, showed the opposite pattern; foragers in this species have lower carbohydrate reserves, and do not show reduced lipid stores. If *F. fusca* workers require a carbohydrate intake to convert into lipid storage, depletion in corporeal carbohydrates, rather than lipids, might act as a cue for an individual worker to leave the nest in search of nectar or other food sources. This result would support the seasonal patterns of foraging preference and

nutrient depletion in a previous study of *P. ceres*, a species that caches seeds but also consumes carbohydrates and proteins. Judd (2006) found evidence that the corporeal levels of carbohydrates and amino acids in foragers vary throughout the year and are negatively correlated with the seasonal foraging preferences for carbohydrates and proteins. A clever study by Mayack and Naug (2013) manipulated the individual energetic state of honeybees and demonstrated that energetically stressed bees increase their foraging rate. Together, these studies support the hypothesis that regulatory mechanisms in solitary insects (responses to individual energetic states or individual nutrient stores) may have been co-opted to drive cooperative foraging behavior in social insect colonies (Mayack and Naug 2013).

One limitation of the current study is that individual protein and amino acid levels were not measured, so we were not able to test the prediction that a preference for protein-based resources (i.e., *Solenopsis* species) is linked to task-specific depletion in proteins or amino acids. This prediction was partially supported for *S. invicta* in an earlier study showing that fire ants collected more insects when protein levels were low (Ricks and Vinson 1972).

#### Resource depletion in external, non-foraging sub-castes

One surprising result from this study is that foragers are not the only workers that exhibit reduced nutrient stores in the colony. To better understand the role of external environment and/or distance from the brood on nutrient stores, we included additional sub-castes in the analysis for polymorphic species. All three species with morphologically distinct sub-castes showed lower nutrient levels in external, non-foraging workers relative to foragers and/or brood care workers. *Camponotus floridanus* is famously sugar biased (Morel et al. 1987) and displays lower stores of both carbohydrates and lipids in major workers relative to minor workers. In this species, minor workers are responsible for most of the foraging while major workers are soldiers, who may forage occasionally. Because we did not have access to brood care workers from this species, it is difficult to assess whether both minor and major sub-castes have lower nutrient stores relative to brood care workers or whether *C. floridanus* would be an exception to the patterns seen in other ant species.

The depressed nutrient stores found in non-foraging external workers suggest that low nutrient stores do not only act as a trigger for foraging behavior as described in other social insects (Kaun et al. 2008; Toth et al. 2005) but may also be associated with the environment or activity levels of workers. There is little evidence to suggest that individual energy states of morphologically distinct sub-castes result from age-dependent decreases in nutrient stores because

majors and soldiers do not necessarily differ in age from conspecific foragers in these species. Another consideration is that the individuals with the lowest nutrient stores are also the largest individuals studied in each species. In all three species, majors are larger than the foraging workers, implying an inverse relationship between size and nutrient stores (Blanchard et al. 2000). A potential alternative explanation for the differences seen between minor and major workers may involve the behavioral flexibility of major workers. Though sub-castes do not transition between behaviors in a consistent or systematic way, under some circumstances, individuals can shift to perform select other tasks. While major *C. floridanus* workers can switch to foraging behavior, minor workers do not perform soldier duties. Given that major workers in some *Pheidole* spp. are also known to be able to transition between tasks (Wheeler and Buck 1992; Lucas and Sokolowski 2009) and the observational evidence that large, soldier-like workers in *Atta* assist in the transport and retrieval of especially large sections of leaf (Wilson 1983), it could be interpreted that low nutrient stores are actually associated with behavioral flexibility rather than foraging behavior. Interestingly, an earlier study of *P. pallidula*, a myrmicine ant with discrete major and minor sub-castes, found variation in nutrient levels and tasks within the major sub-caste (Lachaud et al. 1992). Lean majors were involved in nest defense and majors with distended abdomens were restricted to the nest and acted as storage vessels for lipids. In this species, at least, it appears that the large size of majors is not necessarily correlated with nutrient depletion or task propensity.

Our results in ants indicate that low nutrient stores are linked to external activities in general, including foraging, lending support to the longstanding notion that lean, external workers act as ‘disposable castes’; high-risk tasks outside of the nest are performed by the least energetically costly workers (Porter and Jorgensen 1981; O’Donnell and Jeanne 1995; Blanchard et al. 2000). A number of alternative ecological and life history strategies may factor into nutrient storage patterns; whether species utilize within-nest colony food caches (hives, seed stores, etc.), colony size, differences in preferred metabolic pathways, differential longevity of worker sub-castes, and differences in use of foraging cues (actual vs. perceived). Future studies incorporating diverse species of social insects with behavioral division of labor should help elucidate the correlates of nutrient storage with task behaviors. For example, it is intriguing to consider why two closely related, sympatric species of desert ants that share similar environments might have evolved different physiological responses to metabolic demands (Hahn 2006). It is possible that even within the same ant species, colonies may differ in their collective and individual nutrient storage properties, but this has yet to be rigorously tested.



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## References

- Blanchard GB, Orledge GM, Reynolds SE, Franks NR (2000) Division of labour and seasonality in the ant *Leptothorax albipennis*: worker corpulence and its influence on behaviour. *Anim Behav* 59(4):723–738. doi:10.1006/anbe.1999.1374
- Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37(8):911–917
- Cook S, Wynalda R, Gold R, Behmer S (2011) Macronutrient regulation in the Raspberry crazy ant (*Nylanderia* sp. nr. *pubens*). *Insect Soc* 59:1–8
- Daugherty THF, Toth AL, Robinson GE (2011) Nutrition and division of labor: effects of foraging and brain gene expression in the paper wasp *polistes metricus*. *Mol Ecol* 20:5337–5347
- Fénéron R, Durand JL, Jaisson P (1996) Relation between behaviour and physiological maturation in a Ponerine ant. *Behaviour* 133:791–806
- Fewell JH (1988) Energetic and time costs of foraging in harvester ants, *Pogonomyrmex occidentalis*. *Behav Ecol Sociobiol* 22:401–408
- Gordon DM (1995) The development of an ant colony's feeding range. *Anim Behav* 45:649–659
- Gronenberg W, Heeren S, Hölldobler B (1996) Age-dependent and task-related morphological changes in the brain and the mushroom bodies of the ant, *Camponotus floridanus*. *J Exp Biol* 119:2011–2019
- Hahn DA (2006) Two closely related species of desert carpenter ant differ in individual-level allocation to fat storage. *Physiol Biochem Zool* 79(5):847–856
- Hölldobler B, Wilson EO (1990) *The ants*. Harvard University Press, Cambridge
- Judd TM (2006) Relationship between food stores and foraging behavior of *Pheidole ceres* (Hymenoptera: Formicidae). *Ann Entomol Soc Am* 99:398–406
- Kaun KR, Chakabarty-Chatterjee M, Sokolowski MB (2008) Natural variation in plasticity of glucose homeostasis and food intake. *J Exp Biol* 211:3160–3166
- Kondoh M (1968) Bioeconomic studies on the colony of an ant species, *Formica japonica* Motschulsky. 2. Allometric study of the body mass and corpulency relating to body size of workers. *Jap J Ecol* 64:370–392
- Lachaud JP, Passera L, Grimal A, Detrain C, Beugnon G (1992) Lipid storage by major workers and starvation resistance in the ant *Pheidole pallidula* (Hymenoptera, Formicidae). In: Billen J (ed) *Biology and evolution of social insects*. Leuven University Press, Leuven
- Lucas C, Sokolowski MB (2009) Molecular basis for changes in behavioral state in ant social behaviors. *Proc Natl Acad Sci USA* 106(15):6351–6356
- MacKay WP (1983) Stratification of workers in harvester ant nests (Hymenoptera: Formicidae). *J Kansas Entomol Soc* 56:538–542
- Martin MM, Lieb TJ (1979) Patterns of fuel utilization by the thoracic muscles of adult worker ants: the use of lipid by a Hymenopteran. *Comp Biochem Physiol* 64B:387–390
- Mayack C, Naug D (2013) Individual energetic state can prevail over social regulation of foraging in honey bees. *Behav Ecol Sociobiol* 67:929–936. doi:10.1007/s00265-013-1517-6
- McGlynn T (1999) The worldwide transfer of ants: geographical distribution and ecological invasions. *J Biogeogr* 26(3):535–548
- Morel L, Vander Meer RK, Lavine BK (1987) Ontogeny of nestmate recognition cues in the red carpenter ant (*Camponotus floridanus*). *Behav Ecol Sociobiol* 22:175–183
- O'Donnell S, Jeanne RL (1995) Worker lipid stores decrease with outside-nest task performance in wasps: implications for the evolution of age polyethism. *Experientia* 51:749–752
- Pereira HS, Sokolowski MB (1992) Mutations in the larval foraging gene affect adult locomotory behavior after feeding in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 90:5044–5046
- Porter SD, Jorgensen CD (1981) Foragers of the harvester ant, *Pogonomyrmex owyheeii*: a disposable caste. *Behav Ecol Sociobiol* 9:247–256
- Porter SD, Sanford D, Tschinkel WR, Walter R (1987) Foraging in *Solenopsis invicta* (Hymenoptera: Formicidae): Effects of weather and season. *Environ Entomol* 16(3):802–808
- Ricks BL, Vinson SB (1972) Changes in nutrient content during one year in workers of the imported fire ant. *Ann Entomol Soc Am* 65:135–138
- Risch SJ, Carroll CR (1982) Effect of a keystone, predacious ant, *Solenopsis geminate*, on arthropods in a tropical agroecosystem. *Ecology* 63(6):1979–1983
- Robinson GE (1987) Regulation of honey bee age polyethism by juvenile hormone. *Behav Ecol Sociobiol* 20:329–338
- Robinson GE (1992) Regulation of division of labor in insect societies. *Ann Rev Entomol* 37:637–665
- Robinson GE, Page RE, Strambi C, Strambi A (1989) Hormonal and genetic control of behavioral integration in honey bee colonies. *Science* 246(4926):109–112
- Seid MA, Traniello JFA (2006) Age-related repertoire expansion and division of labor in *Pheidole dentata* (Hymenoptera: Formicidae): A new perspective on temporal polyethism and behavioral plasticity in ants. *Behav Ecol Sociobiol* 60(5):631–644
- Smith CR, Toth AL, Suarez AV, Robinson GE (2008) Genetic and genomic analyses of the division of labour in insect societies. *Nat Rev Genet* 9:735–748
- Smith CR, Suarez AV, Tsutsui ND, Wittman SE, Edmonds B, Freauff A, Tillburg CV (2011) Nutritional asymmetries are related to division of labor in a queenless ant. *PLoS One* 6(8):22401
- Stradling DJ (1987) Nutritional ecology of ants. In: Slansky F, Rodriguez JG (eds) *Nutritional ecology of insects, mites, spiders and other related invertebrates*. John Wiley and Sons Inc, New York, pp 927–970
- Toth AL, Robinson GE (2005) Worker nutrition and division of labor in honey bees. *Anim Behav* 69(2):427–435
- Toth AL, Robinson GE (2007) Evo-devo and the evolution of social behavior. *Trends Genet* 23(7):334–341
- Toth AL, Kantarovich S, Meisel AF, Robinson GE (2005) Nutritional status influences socially regulated foraging ontogeny in honey bees. *J Exp Biol* 208:4641–4649
- Tschinkel WR (1993) Sociometry and sociogenesis of colonies of the fire ant *Solenopsis invicta* during one annual cycle. *Ecol Monog* 63:425–457
- Tschinkel WR (1998) Sociometry and sociogenesis of colonies of the harvester ant, *Pogonomyrmex badius*: worker characteristics in relation to colony size and season. *Insect Soc* 45(4):385–410
- Vinson SB (1968) The distributions of an oil, carbohydrate and protein food source to members of the imported fire ant colony. *J Econ Entomol* 61:712–714
- Wallis DI (1962) Behavior patterns in the ant, *Formica fusca*. *Anim Behav* 10:105–108

- Wheeler DE, Buck NA (1992) Protein, lipid and carbohydrate use during metamorphosis in the fire ant, *Solenopsis xyloni*. *Physiol Entomol* 17:397–403
- Wheeler DE, Martinez T (1995) Storage proteins in ants (Hymenoptera:Formicidae). *Comp Biochem Physiol* 112:15–19
- Wilson EO (1975) *The insect societies*. Belknap Press of Harvard University Press, Cambridge
- Wilson EO (1983) Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: Atta). *Behav Ecol Sociobiol* 14(1):47–54
- Zenner-Polanía I (1990) Management of the “Hormiga Loca” *Paratrechina (Nylanderia) fulva* (Mayr). *Appl Myrmecol World Perspect* 701–707