

# Relationship Between Food Stores and Foraging Behavior of *Pheidole ceres* (Hymenoptera: Formicidae)

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**ABSTRACT** In temperate climates, animals that produce long-term food caches must forage simultaneously for nutrients to survive, reproduce, and collect enough stores to survive the winter. Thus, an animal is simultaneously makes two foraging choices 1) what nutrients to collect for its immediate needs and 2) what food items should be stored. The cues the animal uses to make these two decisions should differ. This study examined the nutrient content of a seed-caching ant, *Pheidole ceres* (Wheeler) (Hymenoptera: Formicidae), to determine whether the nutrients found in stored seeds were different from the cues used to forage. The quantity of carbohydrates, lipids, amino acids, and proteins was measured in individual ants and seeds taken from eight *P. ceres* field colonies once a month from April to October. The relative nutrient levels were compared with the known foraging preferences of *P. ceres* at different times of the year. Seeds serve as the primary lipid and protein storage vessels, whereas the workers store amino acids and carbohydrates. The levels of carbohydrates and amino acids matched the foraging preferences for carbohydrates and proteins, respectively. This pattern suggests that the ants use nutrients that are not abundant in seeds as foraging cues.

**KEY WORDS** *Pheidole ceres*, ants, food storage, foraging, seeds

IDEALLY, ANIMALS LIVING IN temperate zones reproduce when climate is favorable for foraging and focus on survival and food storage when climate becomes unfavorable (Pohl 1976, Lucas 1989). Protein intake is increased when animals reproduce, and carbohydrate and lipid intake increases when animals store food.

Animals that depend on products of plant reproduction (nectar, pollen, and seeds) are exploiting a food item that is itself taking advantage of favorable climate to reproduce and grow. For these animals, reproductive seasons are usually the best times to acquire food for storage. Individuals dependent on seeds, fruit, and nectar should maximize reproduction during favorable times and budget sufficient time and energy for food storage.

The effects of food storage on foraging strategies are well documented in rodents and birds. Hibernating rodents and migrating birds that do not cache seeds switch between food high in protein and food high in lipids and carbohydrates when alternating between reproduction and storage (Pohl 1976, Lucas 1989, Hill and Florant 1997). However, seed-caching rodents and birds feed and store food throughout the year (Vander Wall 1990, Steele et al. 1996, Steele and Koprowski 2001). In these cases, foragers have mechanisms in place that allow them to decide whether to store or eat a food item.

Social insects face the same issues as solitary foragers. Colonies are made up of several to thousands of individual units, each making decisions based on cues they perceive. The sum of these individual decisions results in colony level behavior. Thus, “colony decisions” and “colony needs” are emergent properties stemming from the sum of the behaviors and needs of a colony’s individual units. As a result, the emergent behaviors of a colony should mimic what an individual animal would do in a similar environment.

As with some vertebrates, many social insects toggle between reproduction and storage. Annual colonies such as social wasps and bumblebees initiate new colonies each year (Wilson 1971, Michener 1974). Initially, foragers from the colonies collect protein sources and high-energy foods (carbohydrates and lipids) to rear new workers and reproductives. Once the reproductive adults emerge, the colonies switch their focus to collecting high-energy foods (nectar), presumably to enable reproductive females to build up food reserves for overwinter survival (West-Eberhard 1969, Hoshikawa 1981, Tsuchida 1991, O’Donnell 1998).

Seed-harvesting ants and honey bees engage simultaneously in food storage and reproduction. Honey bees collect excess pollen and nectar, much of which is stored in cells, to ensure colony survival during low productive periods (Waddington 1987, Seeley 1997). Ants do not construct specialized storage containers but instead have other internal and external mecha-

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nisms for food storage. All ants are able to store nutrients internally in their crops, fat bodies, and in the form of protein stores (Stradling 1987). Certain ant species collect seeds throughout the colony's active season (Mehlhop and Scott 1983, Gordon 1991) and use seeds as a food storage vessel (Hölldobler and Wilson 1990, Johnson 2000). How seed collection affects internal food stores and subsequently the foraging behavior of seed-harvesting ants has yet to be explored.

In most food-caching animals, the cues that regulate hoarding behavior are distinct from those that regulate immediate food choices (Vander Wall 1990). Because the colony as a whole is involved simultaneously in several foraging strategies, there should be some mechanism for individual foragers to assess quality of a food source and to decide what to store. In food-caching colonies, this mechanism should 1) allow the colony to allocate its limited foraging force efficiently so that only needed nutrients are gathered and 2) use independent cues for caching decisions and assessment of food quality.

Worker ants do not respond directly to the needs of the larvae (Cassill and Tschinkel 1995). Instead, workers must rely on some other cue to determine what nutrients to gather. One possibility is the nutritional stores within the ants themselves (Blanchard et al. 2000). It has been shown that levels of protein and lipids vary within individuals during different stages of development (Wheeler and Buck 1992) as well as during different times of the year (Vinson 1968, Tschinkel 1993). Because these levels vary, they are potential cues for workers to assess the nutritional status of the colony. Blanchard et al. (2000) found that the lipid levels in *Leptothorax albipennis* (Curtis) corresponded to foraging behavior. Foragers had lower levels of lipids than nonforagers. In seed-harvesting ants, nutrients stored by the colony are located not only in their internal stores but also in the seeds collected. Thus, seed-caching ants should rely only on nutrients that are not abundant in the seeds because an individual ant is unable to determine the number of seeds stored by the colony.

I examined the nutritional content in colonies of the seed-caching ant *Pheidole ceres* (Wheeler) (Hymenoptera: Formicidae). I examined the amount of lipids, protein, free amino acids, and carbohydrates in individual ants and seeds taken from the same colony at different times of the year. I used these data to 1) determine whether the nutrients not abundant in seeds tend to fluctuate in the ants' internal stores more than nutrients found in seeds, and 2) I compared these results to foraging behaviors observed by Judd (2005) to determine whether ant nutrient levels correlate with ant foraging behavior. Judd (2005) demonstrated that *P. ceres* changes its food preferences during different times of the year. He found no preference for protein or carbohydrates during the spring, but in summer and fall, carbohydrates were preferred over protein. The current study identifies nutrients being stored by *P. ceres* at different times of the year and

elucidates possible cues foragers use to make foraging decisions.

## Materials and Methods

**Study Organism.** The colonies of *P. ceres* used in this study are found in the Rocky Mountains (Larimer County, Colorado) at  $\approx 2,285$  m above sea level. *P. ceres* colonies go through three major stages during the year: 1) production of sexuals and workers (larvae are reared from April–May; pupae are present through June), 2) care of adult sexuals (adult sexuals remain in the colony throughout July), and 3) rearing of worker larvae (August–October). For more details on the natural history of *P. ceres*, see Judd (2005).

**Collections.** Small samples of ants were collected with an aspirator from 10 wild colonies and frozen at  $-80^{\circ}\text{C}$ . I collected two majors, six to 10 minors, and a few adult sexuals, when present, from each colony on the first of the month from April 2001 to October 2001. These numbers were chosen to minimize any impact on the colony.

Seeds were gathered randomly as ants were being aspirated. Seeds were separated by morphological traits and frozen at  $-80^{\circ}\text{C}$ . Seeds with multiple representatives in the collections were identified to species.

**Ant Analyses. General Procedures.** Head width and pronotum width were measured on each ant to estimate body size (Blanchard et al. 2000). Wet weight of each ant was determined. The dry weight was not measured because the ants were tested further for nutrients. Individuals were homogenized in  $150\ \mu\text{l}$  of distilled water and centrifuged at 14,000 rpm. Four nutrients types were measured (see below) in the following order: protein, free amino acids, carbohydrates, and lipids. All individuals from a single colony were analyzed simultaneously to minimize between-trial variance.

**Soluble Protein and Free Amino Acids.** I used the florescamine assay (Böhlen et al. 1973) to estimate total soluble proteins and total free amino acids as follows: 1)  $50\ \mu\text{l}$  of the aliquot was taken; 2) each sample was split into four equal portions; 3)  $12.5\ \mu\text{l}$  of 5% trichloroacetic acid (TCA) was added to two of the portions, to precipitate the proteins and to determine total free amino acids; and 4)  $12.5\ \mu\text{l}$  of distilled water was added to the other two portions. These steps provided a measure of total amine containing groups that included proteins and total free amino acids. Because of the sensitivity of this assay, I cut all measurements by half when analyzing majors and males and by fourfold when analyzing gynes. To all samples (regardless of caste), I added 1.45 ml of 0.1 M boric acid and then mixed in 0.5 ml of Fluoran (0.3 mg of fluorescamine/ml of acetone). Each sample was measured on a fluorometer at 485 nm (excitation filter 390 nm and no emission filter). The samples were compared with a 40-nmol standard ( $982\ \mu\text{l}$  of 5%TCA +  $8\ \mu\text{l}$  of 10 mM tyrosine in 10% TCA). For each ant, the average of the measurements for total free amino acids was subtracted from average measurement of the total

amine groups to provide a measure of total soluble proteins.

**Carbohydrates.** Total carbohydrates were measured with the anthrone assay (Van Handel 1985, Wheeler and Buck 1992) as follows: 1) to 50  $\mu\text{l}$  of ant aliquot, 12.5  $\mu\text{l}$  of 18%  $\text{Na}_2\text{SO}_4$  and 1.25 ml of anthrone reagent was added; 2) the sample was heated at 100°C for 12 min and then cooled to room temperature; and 3) the absorbance of the sample was measured at 625 nm with a spectrophotometer and compared with the absorbance of 0, 5, 10, 15, 25, 50  $\mu\text{g}$  of glucose/ $\mu\text{l}$  of  $\text{H}_2\text{O}$  standards. This procedure provided an estimate of micrograms of carbohydrates per ant.

**Lipids.** Total lipids were measured with the phosphovanillin assay described by Barnes and Blackstock (1973), Wheeler and Buck (1992), and Wheeler and Buck (1992) as follows: 1) 50  $\mu\text{l}$  of chloroform/methanol (1:1) was added to the remaining ant sample; 2) the mixture was vortexed and centrifuged for 2 min at 14,000 rpm; 3) the chloroform portion was separated from the rest of the sample and dried; 4) the water-soluble half was added to the dried sample in case any lipids were left behind; and 5) to each sample, 200  $\mu\text{l}$  of concentrated sulfuric acid was added and the sample was heated at 100°C for 10 min. Once removed, 3 ml of vanillin reagent was added, and the sample was allowed to develop for 30 min. The absorbance was measured at 525 nm on a spectrophotometer and compared with the absorbance of 0, 18, 45, 72, and 90  $\mu\text{g}$  of corn oil in chloroform/methanol (1:1) standards (Barnes and Blackstock 1973, Wheeler and Buck 1992).

**Seed Nutrient Analysis.** Nutrient content of seeds harvested commonly by the *P. ceres* was measured with the same procedures as the ants except for two changes. First, I used separate seeds for each analysis. Second, I homogenized the seeds with a Dremel tool with a 1.5-mm wood-carving bit. Sample sizes varied from species to species because the collected seeds varied in both size and number.

**Data Analysis.** The data were analyzed in two ways. First, for each caste, the mean and 95% CI for each nutrient, corrected for mass, was determined for each month. This analysis gave an overall idea of how nutrients changed over time (Fig. 1).

Second, because individual ants came from one of 10 colonies and nutrient levels were not necessarily independent, I used principal components analysis (PCA) as a data reduction tool and as a means of ensuring independence of the resultant axes. One PCA was applied to log-transformed physical data (mass, head width, and pronotum width), and another PCA was applied to log-transformed nutritional data. Each PCA was based on the covariance matrix of the original variables (physical and nutrient measurements) and was orthogonal to the other axes. Each axis was defined clearly in terms of the original variables. Eigenvectors derived from the PCA were used in a canonical correlation analysis (CCA) in an effort to identify patterns relating nutrition to physical properties of the ants. In an effort to identify temporal effects, the principal component scores derived from

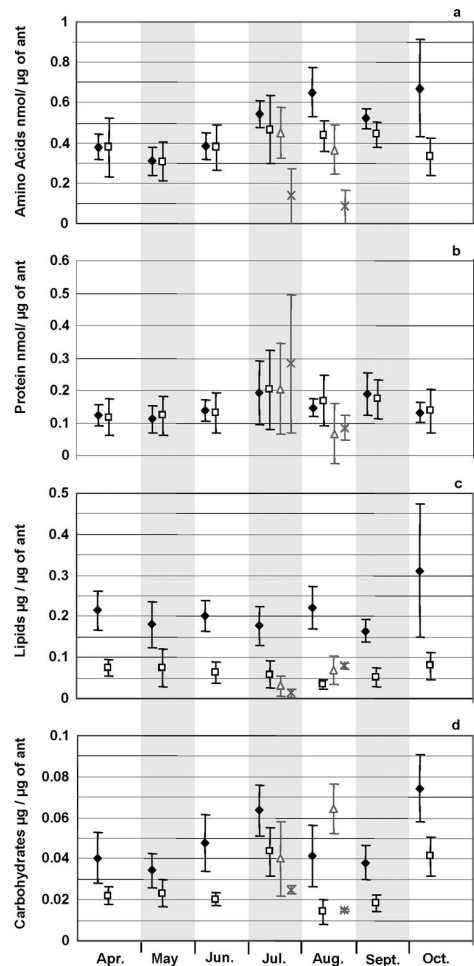


Fig. 1. The 95% CIs depicting the monthly levels of (a) free amino acids, (b) protein, (c) lipids, (d) carbohydrates per microgram of ant in *P. ceres* minors (black diamonds), majors (open squares), female reproductives (open triangles), and males (x) collected during the colony's active season.

the nutrient analysis were used in a stepwise regression model by using the principle component scores from the physical properties, each month, and each colony as predictor variables. Majors and minors were analyzed separately. Because the number of adult reproductives was small, I compared their nutrient changes between July and August by using the Wilcoxon signed rank test.

**Between Caste Comparisons.** The differences in the amount of nutrients per unit mass in minors, majors, males, and gynes were compared. Canonical discriminant functions analysis was used to generate axes that provided maximum separation between groups. This method was used because sample sizes of each caste were different. Scores from the canonical discriminant analysis were extracted and analyzed using an analysis of variance followed by a Tukey's honestly significant difference test to determine differences.

**Table 1. Eigenvectors and cumulative variation from PCAs of the natural log of the four nutrient measurements for both minors and majors**

	Minors				Majors			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
Carbohydrates	-0.0801	0.0493	0.9387	-0.3318	0.0184	0.1543	0.9873	0.0334
Lipids	-0.1481	0.9022	0.0804	0.3972	-0.1568	0.9500	-0.1531	0.2225
Amino acids	0.0303	-0.4001	0.3261	0.8556	-0.3484	-0.2328	0.0041	0.9719
Proteins	0.9853	0.1519	0.0784	0.0064	0.9868	0.1400	-0.0426	0.0691
Cumulative variation	0.5281	0.7370	0.8797	1.0000	0.7194	0.8875	0.9620	1.0000

**Results**

**PCA Results.** For minors, the principal components analysis of the nutritional data produced four eigenvectors (Table 1). The first component was loaded most heavily by proteins and explained nearly 53% of the variance in the nutritional data. This component is thus labeled. The second, third, and fourth eigenvectors were loaded by lipids, carbohydrates, and amino acids and accounted for 21, 14, and 12% of the variance in the data, respectively (Table 1). The PCA for the morphological data on minors produced three axes, with the first dominated by mass (91% of the variance), the second dominated by pronotum width (6.5% of the variance), and the third dominated by head width (2% of the variance; Table 2). Based on these results, I refer to nutrition principal components 1-4 as proteins, lipids, carbohydrates, and amino acids. Similarly, the principal components for body shape are labeled mass, pronotum width (PW), and head width (HW), respectively.

PCA results for ant majors produced eigenvectors dominated by proteins, lipids, carbohydrates, and amino acids (Table 1). These axes are henceforth referred to as proteins, lipids, carbohydrates, and amino acids (AA), respectively. These axes accounted for 72, 17, 7, and 4% of the variance, respectively. For the morphological data, the first component was loaded most strongly by mass, and the second and third axes were loaded by a combination of HW width and PW. The first axis is referred to as mass (Table 2). It accounted for 86% of the variation in the morphological data.

**General Measurements.** The canonical correlation analysis for the minors produced two significant correlations. The first canonical axis for nutrients was dominated by carbohydrates, and the first canonical axis for morphology was dominated by mass. Thus, there is a positive relationship between carbohydrate content of the ant minors and body mass. The second significant canonical correlation was between a pro-

tein axis and HW. However, HW was loaded negatively and thus had reversed polarity. Ant minors with small HW had higher protein content.

The canonical correlation between nutrient and morphology axes from the PCA for majors revealed a significant correlation between ant mass and content of both carbohydrates and amino acids. There was no significant relationship with any of the four nutrient groups and body measurements in gynés and males.

**Changes in Nutrient Levels during the Year.** *Minors and Majors.* Levels of amino acids in minors were low in spring (April and May), rose significantly from May until August, and remained high in the fall (Fig. 1a). Results of the stepwise regression also showed a significant decrease in May ( $F = 7.31, P = 0.0072$ ) and a significant increase in August ( $F = 21.77, P = 0.0001$ ). Majors showed a similar pattern, but instead the levels of amino acids peaked in July (Fig. 1a). There seemed to be a slight drop in amino acids in majors in September, but this drop was not significant. The stepwise regression showed a significant rise in amino acids in July ( $F = 19.22, P = 0.0039$ ) and a slight nonsignificant drop in September ( $F = 2.78, P = 0.098$ ). Overall, it seems both castes are low in amino acids in the spring and increase their stores in the summer and maintain these stores through the fall.

Proteins showed very little change in either minors or majors (Fig. 1b). This finding was confirmed in the stepwise regression because no month showed any significant effects. Thus, there are no significant changes in protein levels in *P. ceres*.

When examining the confidence intervals, lipids showed no significant changes for either caste (Fig. 1c). However, the stepwise regression for minors showed significant peaks in lipids in April ( $F = 16.36, P = 0.0001$ ), June ( $F = 7.00, P = 0.0085$ ), and October ( $F = 6.45, P = 0.012$ ). These peaks suggest that in the absence of colony effects lipids were significantly highest in these 3 mo for minors. Stepwise regression

**Table 2. Eigenvectors and cumulative variation from the PCAs of the natural log of the morphological measurements for both minors and majors**

	Minors			Majors		
	PC 1	PC 2	PC 3	PC1	PC2	PC3
HW	0.1181	0.3972	0.9101	0.2440	0.7331	-0.6348
PW	0.0591	0.9121	-0.4057	0.1855	0.6072	0.7726
Mass	0.9912	-0.1018	-0.0842	0.9519	-0.3063	0.0122
Cumulative variation	0.9119	0.9770	1.0000	0.8519	0.9666	1.0000

**Table 3. Results of the canonical discriminant analysis comparing the nutrient content between different castes**

	CAN 1	CAN 2
Protein	-0.0084	-0.0450
Carbohydrates	0.7878	-0.6025
Amino acids	0.4463	0.0724
Lipids	0.8784	0.4698
Cumulative	0.928	0.988
Likelihood ratio	0.913	0.993
P value	0.0001	0.7813

results for majors showed a significant peak in October ( $F = 5.60, P = 0.02$ ) but no other change.

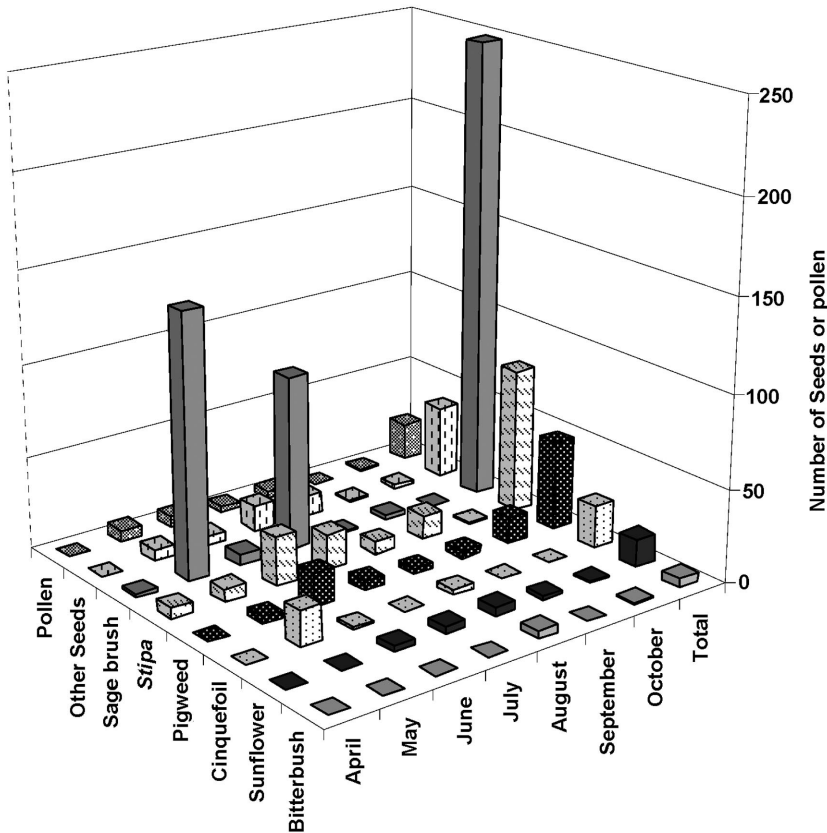
Results for both minors and majors revealed that carbohydrate levels were low in early spring, increased during June, and were significantly highest in July (Fig. 1d). The stepwise regression also showed a significant increase in July for minors ( $F = 47.88, P = 0.0001$ ) and majors ( $F = 19.41, P = 0.0001$ ). Carbohydrate levels decreased significantly between July and August in both castes (Fig. 1d) and increased significantly between September and October (Fig. 1d). The stepwise regression showed significant differences for August (minors:  $F = 4.96, P = 0.027$ ; majors:  $F = 11.62, P = 0.001$ ) and October (minors:  $F = 10.26, P = 0.0015$ ; majors:  $F = 16.84, P = 0.0001$ ).

**Reproductive Adults.** Both reproductive males and reproductive females showed no significant changes in the levels of amino acids or in the levels of protein (Fig. 1a and b). There was a significant increase in lipid content from July to August in reproductive females (Wilcoxon:  $N_{\text{july}} = 4, N_{\text{aug}} = 2, P < 0.05$ ; Fig. 1c). Unlike the female reproductives, the males did not show a significant change in lipid levels (Fig. 1).

The reproductive males showed a significant increase in the amount of carbohydrates from July to August (Wilcoxon:  $N_{\text{july}} = 6, N_{\text{aug}} = 3, P < 0.05$ ; Fig. 1d). However, the reproductive females showed a significant drop in carbohydrates levels from July to August (Wilcoxon:  $N_{\text{july}} = 4, N_{\text{aug}} = 2, P < 0.001$ ; Fig. 1d).

**Between Caste Comparisons.** The results of the canonical discriminant analysis (Table 3) produced one significant axis ( $P = 0.0001$ ) and had a coefficient of determination of 91.3%. Results of the a posteriori test revealed that minors contained significantly more lipids and carbohydrates per unit body mass than majors and sexuals. The other castes did not differ with respect to the four nutrients.

**Seeds. Types of Seeds Collected.** The five species of seeds found more than once in individual colonies of *P. ceres* were from sunflower, *Helianthus annuus* L.;



**Fig. 2.** Number of different seed types or pollen collected at different times of the year from 10 *P. ceres* colonies.

Table 4. Means (SE) of the nutrients per seed found in seeds collected from colonies of *P. ceres*

Seed type	Ncl/Nap	Carbohydrates ( $\mu\text{g}/\mu\text{g}$ seed)	Lipids ( $\mu\text{g}/\mu\text{g}$ seed)	Amino acids (nmol/ $\mu\text{g}$ seed)	Estimated amino acid ( $\mu\text{g}/\mu\text{g}$ seed) <sup>a</sup>	Protein nmol/ $\mu\text{g}$ seed
Sage brush	40/8	0.007 (0.0048)	2.31 (0.34)	2.18 (0.66)	0.30	1.76 (0.64)
Grass	8/4	0.013 (0.0087)	0.92 (0.17)	2.13 (0.33)	0.30	0.00 (0.00)
Pig weed	4/4	0.045 (0.012)	1.41 (0.076)	0.86 (0.13)	0.12	0.68 (0.32)
Sunflower	2/1	0.035 (0.023)	3.05 (2.38)	0.26 (0.00)	0.03	0.18 (0.18)
Cinquefoil	1/1	0.00 (0.00)	1.75 (0.00)	2.63 (0.00)	0.37	0.00 (0.00)

Ncl/Nap, number of seeds used in the carbohydrates and lipids analyses/number of seeds used in the amino acids and protein analysis.

<sup>a</sup> Estimate based on the average mass of all 20 amino acids = 0.1396  $\mu\text{g}/\text{nmol}$ .

needle grass (*Stipa* spp.); cinquefoil, *Potentilla glandulosa* Lindl.; pigweed, *Chenopodium album* L.; and sagebrush, *Artemisia tridentata* Nutt. The quantity of seeds collected in different months is shown in Fig. 2. Sagebrush, needle grass, and pigweed seeds were the predominant seeds collected by the ants (Fig. 2). Cinquefoil seeds were collected in abundance only in May, possibly because this species is a spring-blooming plant and produced seeds only in May (Fig. 2). There were 25 other seed species found in the colonies I examined. These were found only once. Based on my sample, sagebrush seeds were the most common seeds collected by *P. ceres*. Sagebrush is one of the most common plants in this environment, so it is not surprising that the *P. ceres* collected many seeds from this species. The relative abundance of other plants still needs to be assessed to see whether their abundance matches the proportions of seeds found in the colonies.

In addition to seeds, I found many pollen grains (Fig. 2) in the samples. The pollen was primarily from angiosperms, but the actual species of the pollen is unknown. I also found the remains of insects, including ants, beetles, and homopteran nymphs.

**Seed Nutrients.** In all cases, seeds contained a large amount of lipids (Table 4). Seeds of sagebrush, pigweed, and sunflower also contained protein, whereas needle grass and cinquefoil seeds did not contain protein. Sagebrush seeds contained the highest amount of protein relative to their weight than every other seed (Table 4). Based on this analysis, sagebrush seeds are the most nutritious seeds collected by *P. ceres*.

## Discussion

The results indicate that 1) *P. ceres* adult reproductives showed significant changes over time in carbohydrate and lipid content but not in protein or free amino acid content; 2) minors showed significant changes in carbohydrates, free amino acids, and lipids but not proteins; 3) majors showed only significant changes in carbohydrates and amino acids but little change in proteins and lipids; and 4) all seeds commonly collected by *P. ceres* were high in lipids but only two species (sagebrush and pigweed) contained a high amount of protein.

**Nutrient Changes in Adult Reproductives.** The changes in carbohydrate and lipid levels over time were different for adult male and female reproductives. Carbohydrate levels increased in males but de-

creased in female alates. Unlike males, female lipid levels increased during July, suggesting the females converted their carbohydrates to lipids for long-term storage. Males require just enough energy reserves to locate a mate, and do not need long-term stores, thus explaining the static lipid content in males. These findings are consistent with patterns of lipid change in reproductive adults of other ants (Peakin 1972, Passera and Keller 1990).

Although ants use storage proteins (Wheeler and Martinez 1995), the lack of change in proteins or amino acids over time in adult female reproductives was consistent with findings from other studies. Vinson (1968) demonstrated that male and female alates of *Solenopsis invicta* (Buren) gained all of their protein stores as larvae. It is likely larvae of *P. ceres* acquire protein stores as well, which would increase the demand for protein when adult reproductives are reared. Indeed, Judd (2005) demonstrated that the highest preference for protein in *P. ceres* colonies occurred when reproductive-destined larvae were present.

**Nutrient Changes in Workers.** Carbohydrates and amino acids showed greater variation in *P. ceres* workers than lipids and proteins. This result is the opposite from what has been observed in nonseed harvesting ants. Ricks and Vinson (1972) showed that in *S. invicta* both protein and lipids varied much greater than carbohydrates. The main difference between these two ants is *P. ceres* harvests seeds. Protein and lipids are readily available in seeds *P. ceres* collects. This difference suggests workers of seed harvesting ants may forgo lipid and protein storage in favor of smaller nutrients.

**Food Storage.** Food stores in *P. ceres* seem to exist in three forms: long-term storage in seeds, short-term reserves in majors, and immediate reserves in minors. The seed, an adaptation for storing food for a plant embryo, is an ideal long-term food storage vessel. Seeds collected by *P. ceres* fall into two categories: seeds high in lipids and seeds high in protein and lipids. Sagebrush, the most commonly collected seed type, has a higher percentage of protein than all of the other seeds collected and represents 52% of the total seeds sampled. This finding suggests that *P. ceres* is possibly collecting the most nutritious seeds preferentially.

Castes involved in colony care are continuously exchanging food with other colony members (Vinson 1968; Brian and Abbott 1977; Cassill and Tschinkel 1995, 1999; Cassill et al. 1998; Cassill and Tschinkel 1999). In *Pheidole*, minors are the primary caregivers

(Wilson 1984). In this study, the minors' carbohydrate, amino acid and lipid stores fluctuated quite a bit during the year. Carbohydrates and amino acids are easier to use and are less efficient for storage than proteins and lipids and are probably allocated to other colony members first. Thus, changes in minors' nutrient levels likely represent changes in immediate needs of the colony. Lipids fluctuations might only occur when colonies are nutrient stressed.

There were two time periods in which colonies were nutritionally stressed in the spring (April and May) and midsummer (July and August). In the spring, minors had low levels of carbohydrates and amino acids. Two factors could have contributed to these low levels in nutrients: 1) the colony was emerging from overwintering and 2) both reproductive and worker larvae were being reared. Lipid levels were high in April but soon dropped in May. The lipids may have been available from the seed stores; however, the colonies were potentially stressed enough where most of the minors' lipid reserves were drained.

From July to August, both males and female reproductives increased their energy reserves during their stay in the colony. The sharp decrease in carbohydrate levels in the minors and majors from July to August, and the significant drop in lipid stores in minors suggests that feeding of adult sexuals can drain a colony's carbohydrate and lipid stores. A similar drop in lipids during the production of sexuals was found in *Pogomyrmex badius* (Latreille) (Tschinkel 1998) and *S. invicta* (Tschinkel 1993). Interestingly, in *P. ceres* colonies there were lower numbers of larvae when reproductive adults were present (Judd 2005). The lack of larvae, the main protein and amino acid sink (Weeks et al. 2004), was consistent with the maintenance of high amino acid levels in the colony from July to August. During July, *P. ceres* colonies seemed to focus on reproduction, whereas growth seemed to be suspended.

There also were two periods in which workers recovered their nutrient stores, early summer (June) and in the fall (September and October). Workers may have been able to regain their amino acid, carbohydrate, and lipid stores during June because of two events. First, the larvae from the spring were pupating relaxing the need for nutrients and second there was an influx of sagebrush and cinquefoil seeds in May, which may have allowed the workers to replenish their lipid stores. In the fall (September and October), the reproductives left and carbohydrate and lipid levels rose once again. The colonies have reproduced and began to prepare for winter. Amino acid levels remained high for the rest of the active season, suggesting that the fall larvae are not able to drain these stores. Thus, *P. ceres* colonies were nutritionally stressed when the reproductive larvae or adults were feeding and recovered during periods when they were not feeding.

The majors' carbohydrate and amino acid levels fluctuated in the same way as minors. The main difference between the two classes was that majors showed a peak in amino acid content in July, whereas

minors peaked in August. This suggests that majors are storing amino acids sooner than minors. There are many species of ants in which majors act as food repletes (Wilson 1974, Lachaud et al. 1992, Tschinkel 1993). In *S. invicta*, *Leptothorax*, and *Camponotus*, the largest individuals (majors if present) contain significantly larger amounts of lipids relative to their size than other colony members. In this study, majors of *P. ceres* did not have significantly higher percentages of lipids than minors. In fact, minors had significantly more nutrients per unit mass than any other caste. Majors seem to be storing food, as evidenced by the fluctuations in amino acids, and carbohydrates as well as the peak in lipid content in October, but not to the large capacity as repletes from other ant species.

The seed-storing behavior of *P. ceres* might explain the discrepancy between lipid content of majors of other ant species and majors of *P. ceres*. Nonseed-storing ants seem to have only two levels of storage: long term and short term. These are carried out by larger and smaller castes, respectively (Wilson 1974, Stradling 1987, Lachaud et al. 1992, Tschinkel 1993). Having majors increase their roles as food stores may buffer nonharvesting ants during a food shortage. If the colony harvests seeds, the role of a major replete becomes redundant. If this hypothesis is true, majors of other seed harvesters also should have a reduced role as food repletes (but see Lachaud et al. 1992).

The reduction of internal lipid stores has been documented in seed-caching vertebrates. Caching species tend to have less fat tissue than related noncaching species. Seed caching birds such as chickadees and titmice have lower lipid reserves than noncaching birds (Lima 1986, Rogers 1987, Hurly 1992, Pravosudov and Grubb 1997). A similar pattern has been observed between seed caching and nonseed-caching squirrels (Vander Wall 1990). The models used to explain the lack of lipids in seed caching vertebrates cite food reserves and predation risk as the major reasons for this behavior (Lima 1986, Lucas and Walter 1991). Heavier animals are presumably slower and more susceptible to predation. Whether seed caching allows vertebrates to store other nutrients more efficiently remains to be tested.

**Relation of Internal Food Stores to Foraging Behavior.** Food storage has potential to affect foraging behavior. Blanchard et al. (2000) found evidence that in the ant *L. albipennis*, nutrient levels may stimulate foraging. In their study, foragers had lower lipid levels than nonforagers. They suggested that low lipid levels stimulated individuals to forage. However, lipids would not be a useful cue for seed storing ants because much of the colony's lipid stores are in seeds and seed number would be hard for a worker to estimate.

Levels of nutrients not found in seeds could provide better cues to colony hunger for seed storing ants. Judd (2005) found that *P. ceres* forages for protein and carbohydrates equally in the spring but prefers carbohydrates in the fall. In this study, carbohydrates and amino acid levels low in spring and amino acid levels were high in fall, but carbohydrates were low. Thus, protein preference was negatively correlated with

amino acid level, and carbohydrate preference was negatively correlated with carbohydrate levels. The seeds collected by *P. ceres* were low in amino acids and carbohydrates, allowing these nutrients to be better indicators of the immediate nutritional status of the colony. Thus, ant workers might cue in on different nutrients depending on their food storage method.

Seed harvesting has two potential consequences: 1) workers may forgo lipid and protein storage in favor of smaller nutrients and 2) demand for protein and lipids by a colony is difficult to determine by an individual because ants cannot estimate seed number. These consequences would favor smaller nutrients (carbohydrates and amino acids) as foraging cues. Nonseed-harvesting ants store all their lipids and proteins internally so these nutrients would be reliable indicators of colony needs. Carbohydrates are probably used immediately by nonseed-harvesting workers or quickly turned to lipid reserves. Thus, food storage method used by an ant species could be used to predict what nutrients fluctuate. Nonseed harvesters should show greater variation in lipids and protein, whereas seed harvesters should show greater variation in amino acid and carbohydrate content. If this hypothesis is correct, and if ants use nutrient levels to make foraging decisions, different species might cue in on different nutrients depending on their life history. This prediction is supported by (Ricks and Vinson 1972). They examined protein, lipid and carbohydrate content of *S. invicta* colonies over a year. Indeed, protein and lipid levels showed more variation than carbohydrate levels. The authors noted that the ants gathered many insects when protein content was low, supporting the idea that nonharvesting ants might cue onto their internal protein and lipid stores.

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