DISSERTATION

THE NUTRITIONAL ECOLOGY OF THE ANT, *PHEIDOLE CERES*

Submitted by
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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY TIMOTHY M. JUDD ENTITLED THE NUTRITIONAL ECOLOGY OF THE ANT, *PHEIDOLE CERES* BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION
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I examined the nutritional ecology of the ant *Pheidole ceres*, a small ant living in the Rocky Mountains, where the seasons range from hot summers to cold winters. If colonies behave the same as solitary organisms, then in this climate, one should expect 1) colonies *P. ceres* to reproduce during warmer months when food is abundant and store food when the colder months approach to prepare for times when food is absent. 2) The colonies should change their nutritional intake to match their strategy. While reproducing, animals take in more protein and when preparing for winter, change their dietary intake to high-energy foods.

Field colonies presented with a choice between protein and carbohydrate sources foraged for both nutrients equally in the spring. At this time, the colony was rearing worker and reproductive destined larvae. In mid-summer, when adult reproductives were present, colonies preferred carbohydrates. This preference was maintained in the fall, when there were only worker-destined larvae. Thus, *P. ceres* colonies showed the greatest preference for protein when the colony was reproducing. This preference changed to carbohydrates as they fattened up their reproductives and prepared for the upcoming winter.

One possible mechanism that would have produced this change in diet was workers were cuing in on the needs of the larvae. To test this, I created colonies consisting of brood deprived of one nutrient and workers deprived of another. The workers ignored needs of the larvae and responded only to their own needs. Thus, larvae have no influence over worker foraging behavior in *P. ceres*.

P. ceres stores food internally as well as in seeds they collect. I analyzed individual ants and seeds, collected monthly, for the amount of protein, free amino acids, carbohydrates, and lipids. Lipids and proteins showed very little fluctuation over the year. However, the seeds contained lipids and protein, suggesting seeds are the primary source for these nutrients. Amino acids and carbohydrate levels correlated with the ants' foraging preference for protein and carbohydrates intake respectively. These results suggest that *P. ceres* workers are possibly cuing in on their own stores when making foraging decisions.

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To: My Parents Wesley H. Judd and Nancy B. Judd,
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Chapter 1: The nutritional ecology of social hymenoptera

Growth, reproduction and general maintenance: each of these attributes is important to an organism and each has different nutritional requirements. When an organism ingests food, two processes occur simultaneously. First, nutrients consumed are allocated according to the requirements of the abovementioned attributes. Second, this distribution process is regulated by an internal communication system. Hormones and other factors regulate nutrient distribution and utilization. Thus, within organisms there are two processes intertwined, the flow of nutrients through the body and the flow of information regulating the distribution of the nutrients.

When studying an individual organism, it is often difficult to tease apart the proportion of nutrients allocated to growth, reproduction and general maintenance. Eusocial colonies can be used as a model system to examine these very processes. Eusocial colonies are made up of many individuals that make independent decisions based on cues available to them, much like cells in the body. Collectively, these decisions produce emergent properties such that a colony as a whole mimics an individual. Colonies as a whole also allocate resources to growth, reproduction and general maintenance. As a result, colonies also have a flow of nutrients and a flow of information regulating the acquisition and distribution of nutrients.

The advantage of studying nutritional ecology in social insects is that different individuals in a colony specialize on growth, reproduction or general maintenance. It is much easier to measure what individual workers are doing than what individual cells are doing. Visualizing a colony as an individual allows us to predict the nutritional needs of the members of the colony. Because a eusocial colony is analogous to an organism, the

corresponding components in a colony (growth, reproduction and general maintenance) should have the same nutritional requirements as corresponding tissues in an individual.

The different life histories represented by eusocial insects parallels the life histories of individual animals. Thus, different groups of eusocial insects could act as models for animals with similar life histories. Hymenoptera colonies are essentially free-living predators and/or scavengers. These species contain individuals that must leave the protection of the nest and forage for food. In addition, the colony must defend itself against potential predators. Social aphids and thrips colonies are essentially parasites. Colonies of these insects live inside a plant (such as in a gall) and feed on the host's juices (Faith 1979; Crespi 1992; Moran 1992). The soldier caste of aphids and thrips essentially prevents non-relatives from entering the gall and competing with their kin (Foster 1990; Crespi 1992; Moran 1993; Stern 1996). Such colony structure parallels the behavior of the trematode parasite *Echinostoma paraensei*. Within a snail, *E. paraensei* produces a mother redia which grows much faster than other rediae (Joe 1966; Sapp et al. 1998). This individual kills off any competing parasites, allowing its clones to mature in a competition-free environment (Sapp et al. 1998). Termite colonies are essentially decomposers. They either live in dead wood or forage for sources of cellulose-based food (Noirot 1989; Roisin 1996; Shellman-Reeve 1997). Comparing the two life history strategies of termites could provide insight to external and internal decomposers (Shellman-Reeve 1997).

In this paper, I will discuss the nutritional ecology of a colony and relate it to organisms as a whole. I will use social Hymenoptera as the model system because it is the best representative of generalist feeders among eusocial insects. I will discuss 1) the

flow of nutrients through a colony, 2) the flow of information through a colony 3) how the two pathways are integrated by a colony and 4) how these two pathways could have influenced the life history of a colony.

The division of attributes within a colony

Members of a colony can be divided into one of three categories: growth, reproduction and general maintenance. For the purpose of this discussion, the latter category includes foraging, defense, elimination of dead individuals and similar functions. Although these functions are different and may involve different individuals (Wilson 1971; Oster and Wilson 1978; Seeley 1983; Robinson 1988; Robinson 1992), the individuals performing these different tasks should all have similar nutritional requirements.

In a colony, as in an individual, there are components that act as the reproducers . Just as cells in gonads produce gametes, so does the hymenopteran queen reproduce for the colony. When considering the nutrients devoted to reproduction, we have to consider the needs of the queen, the nutrients devoted to raising the gyne and male destined larvae, and the needs of adult males and gynes. Thus, the nutrients needed for reproduction change as the reproductives are reared.

For a colony, growth refers to the increase in colony worker population. This is similar to the production of new cells in individuals (Maynard and Loosli 1969). The nutrients devoted to the growth of a colony are those nutrients given to worker-destined larvae. Although it would appear that growth (worker larvae) and reproduction (sexual larvae) should have the same nutritional needs, the difference between growth and

reproduction is the actual amount of food allocated to the two larval types. Caste determination in female hymenoptera is largely dependent on the amount of food larvae are given (Wheeler 1986). Thus, the nutritional investment per individual is different in the two components.

General maintenance is the function of the adult workers. They are responsible for the everyday maintenance of the colony. From a nutritional perspective, workers can be divided into two categories; foragers, individuals that obtain food from the environment, and caretakers, individuals that distribute the food within the colony. In some species of hymenoptera, these castes usually correspond with age (Seeley 1985; Jeanne et al. 1988; Robinson 1992). In other species such as *Polistes,* these divisions are transitory (O'Donnell 1995, Judd, 2000 #7030). In all cases, the nutrients needed to keep a worker active should be similar regardless of the caste.

One final component associated with organisms found in some colonies is food storage. Perennial individuals such as hibernating animals (Pohl 1976) store excess nutrients to buffer themselves when food is unavailable. Perennial colonies also store food and must shift some of the resources they gather to insure colony survival during food shortages.

Colonies can be divided into components similar to those found in individuals. Each of these components has different nutritional requirements. In the following sections, I will trace what is known about the flow of different types of nutrients through a colony, which causes each of these components to get the nutrients it needs. These pathways are very similar to what is expected in an individual organism.

Flow of protein and amino acids

Proteins and amino acids are essential for growth (increase in cell number) and maintenance (cell replacement) in an individual (Maynard and Loosli 1969). Not all parts of an individual are growing (Maynard and Loosli 1969; Pond et al. 1995); consequently the distribution of proteins and amino acids is uneven throughout the individual (Davidson 1997). Thus, in a colony, workers should distribute proteins preferentially to individuals responsible for growth and reproduction.

There are three main sources of protein for hymenoptera: pollen (Michener 1974; Barth 1991), live prey (Stradling 1978; Detrain and Deneubourg 1997; Richter 2000), and seeds {Whitford, 1978 #7021; Mazhar, 1998 #7039; Judd This Volume}. Bees specialize on pollen; wasps and ants are generalist feeders (Stradling 1978; Detrain and Deneubourg 1997; Richter 2000).

Proteins and amino acids are primarily used for growth and the production of enzymes (Maynard and Loosli 1969; Pond et al. 1995). Thus, the individuals involved in growth, the larvae, should receive the majority of the protein entering the colony. In addition to nutrients necessary for growth, larvae also need to store any nutrients needed for pupation, as pupae do not feed (Markin 1970). Wheeler and Buck (1992) found that pupae of *Solenopsis invicta* showed a 60% reduction in the amount of proteins from the beginning of the pupa stage to the end of the pupa stage. *S. invicta* larvae starved prior to the pupa stage contained 10% less protein and were found to be significantly smaller (Wheeler and Buck 1992). As a result, the demand for protein by larvae should be greater than expected based on their size.

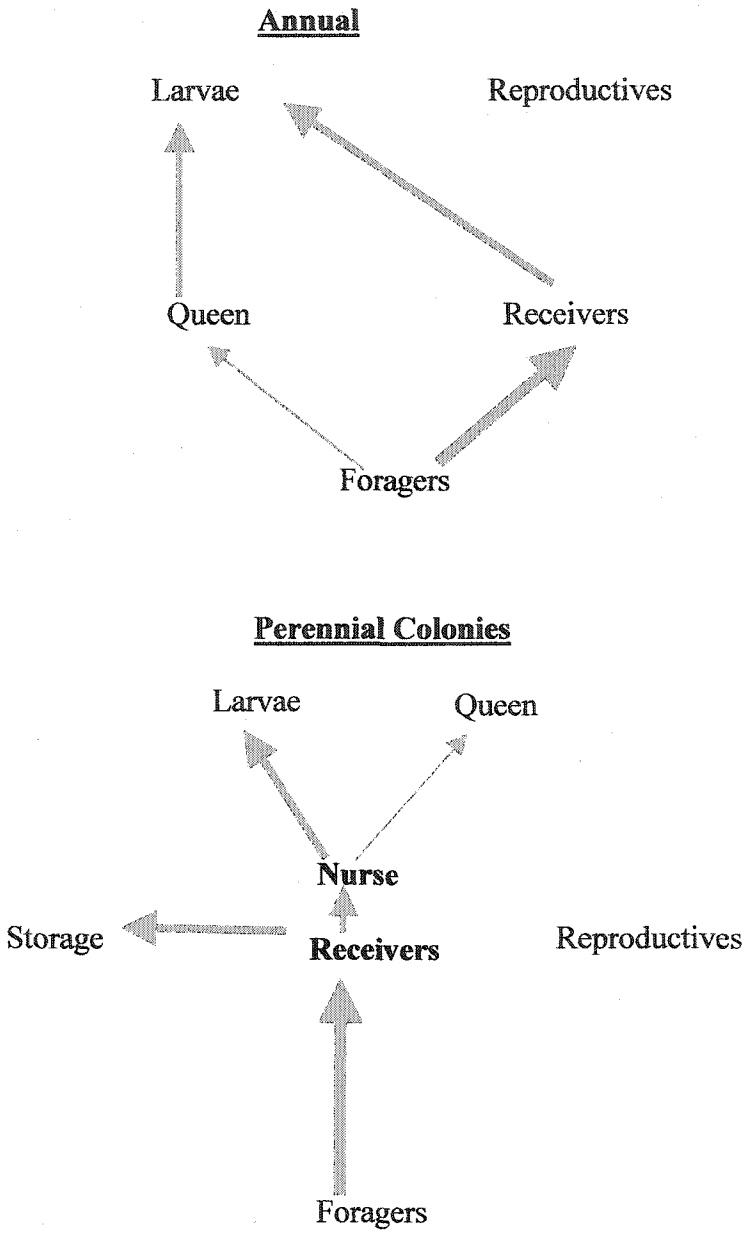
Reproduction also utilizes protein, especially during the production of eggs (Wheeler and Buck 1996). Thus, the queen and female alates should be expected to require protein in their diet. Wheeler (1995) has shown that adult sexuals do contain storage proteins. However, these proteins appear to be acquired during the larval stage. In the ants *Tetramorium caespitum* (Peakin 1972) and *Pheidole ceres* (Judd, This Volume), there are no changes in protein {Peakin, 1972 #7008; Judd This Volume } or amino acid levels (Judd This Volume) in adult sexuals from the time they emerge to the time they fly. This lack of change, suggests that very little protein or amino acids are given to adult sexuals. Reproductive destined larvae not only need proteins and amino acids for growth and pupation as seen in worker destined larvae, but need additional resources for storage proteins.

The demand for nitrogenous nutrients for colonies as a whole should be greatest when reproductive destined larvae are present. In *Pheidole ceres*, the preference for protein is highest during the spring when colonies are raising reproductive destined brood (Judd This Volume). In *Polistes,* caterpillar predation shuts down once the last larvae have pupated (Reeve 1991) and the rest of the season is devoted to collecting nectar. Thus, larvae appear to be the major sink for protein in a colony.

Adult workers, on the other hand, have little need for protein. They do not grow or replace muscle tissue (Kerkut and I 1985). Nitrogen is only needed for enzymes and, in some cases, proteinaceous venoms (Davidson 1997). It is unlikely that the workers retain much of the protein they acquire.

Overall, the flow of protein and amino acids should move through the worker force and be primarily distributed to the larvae (Figure 1). This appears to be the case for

Figure 1: Diagram showing the flow of nitrogenous nutrients through annual and perennial colonies. The thickness in the arrow depicts the relative amount going to each caste.



all Hymenoptera examined so far. Several studies on ants demonstrated that larvae received the majority of the protein. Vinson (1968) has shown that larvae of the fire ant *Solenopsis invicta* received almost all of the protein entering the colony. Cassill and Tshinkle (1999) found that larvae received a large portion of the amino acids gathered by workers. Markin (1970) found that lab colonies of the Argentine ant, *Iridomyrmex humilus*, also donated most of the protein to the larvae. Hunt et al. (1987) demonstrated a similar pattern in colonies of the wasp, *Polybia occidentalis.*

In some hymenoptera, larvae regurgitate food for adults. In wasps, the larval saliva is rich in amino acids (Hunt et al. 1998). Adult wasps have been shown to consume the saliva. This phenomenon has been suggested to occur in bees and ants but the evidence to support this is still sketchy (Hölldobler and Wilson 1990). Markin (1970) found that ants received P^{32} from larvae but it appeared to be from the excretions rather than secretions. If nitrogen is flowing back to the adults from larvae in bees and ants, it may be a minor component in the flow of protein.

Flow of energy compounds: carbohydrates and lipids

Carbohydrates and lipids (specifically fats and oils) serve as energy sources for individuals (Pond et al. 1995). These nutrients are essential for the production of ATP in cells. Such nutrients are constantly distributed throughout the body (Pond et al. 1995). In colonies, energy rich foods such as carbohydrates and lipids are important for all of the members of the colony. Workers need energy in order to perform their tasks while larvae and reproductives need energy for growth and survival.

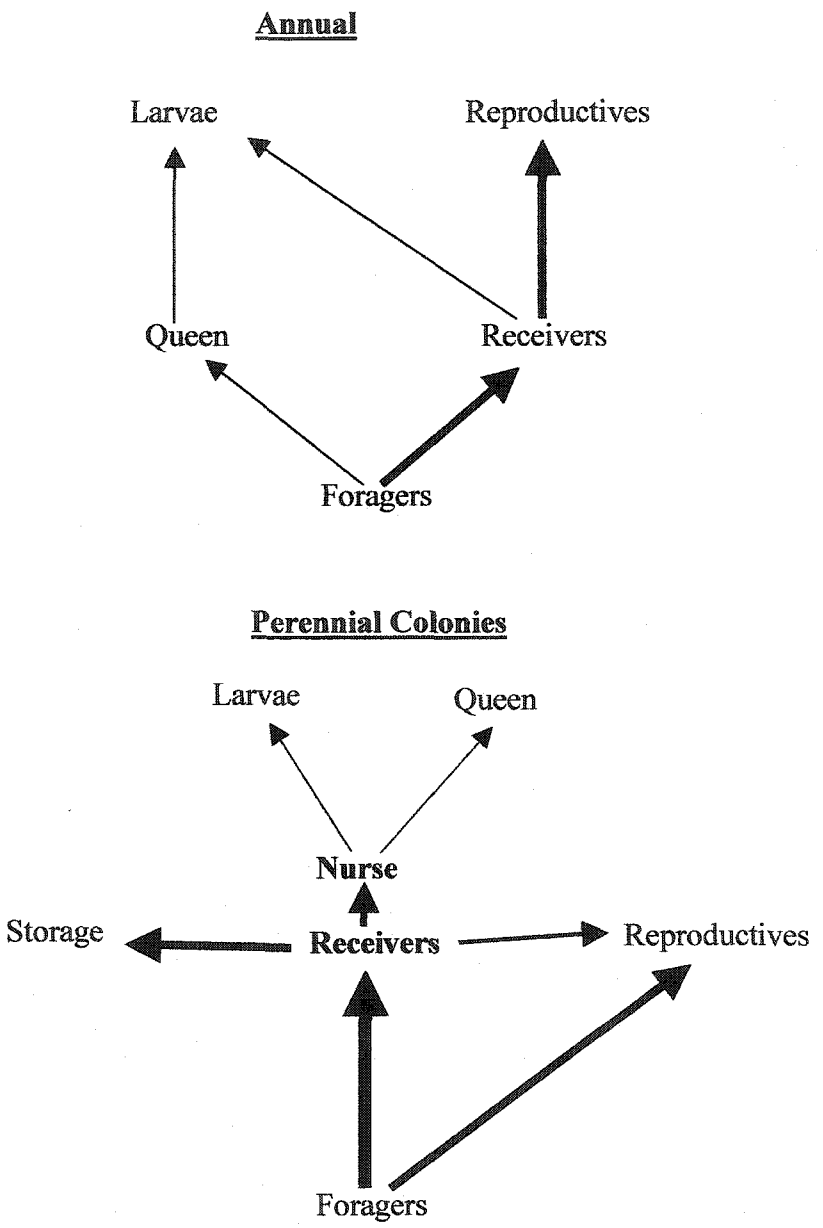
There are several sources of carbohydrates and lipids for Hymenoptera. Carbohydrates are generally gathered from nectar of flowers plant exudates, and in some cases the honeydew excretions of aphids (Stradling 1978; Stradling 1987). Fungus is another rich source of carbohydrates. Martin and Martin (1969) found that the fungus reared by *Atta colombica tonsipes* contains a large amount of carbohydrates (27% of dry weight). In addition, *Atta* contain the enzyme chitinase, allowing them to break down the fungal cell walls (Febvay and Kermarrec 1978). Lipids are obtained from a variety of places. Seeds and prey items are both important sources of lipids for Hymenoptera (Hunt 1991, Stradling, 1987 #6993).

As with cells in an individual, everyone in the colony needs energy. Thus, the flow of carbohydrates and lipids in a colony should be more uniform than the flow of proteins (Figure 2). Carbohydrates are the primary food source for workers. Workers of ants (Vinson 1968; Cassill and Tschinkel 1999), wasps (Hunt et al. 1987; Gamboa 1990) and bees (Michener 1974) all receive a healthy portion of the carbohydrates gathered by the colony.

In an individual, rapidly growing tissues burn more energy than slowly growing tissues (Maynard and Loosli 1969). Similarly, larvae, rapidly growing entities, need large amounts of energy as well. In addition, just as with the protein reserves, larvae need to store enough lipids to survive pupation. As a pupa, an individual ant loses a large amount of its stored lipids (Peakin 1972; Wheeler and Buck 1992).

The other individuals that require energy-rich foods are the adult reproductives (Wheeler and Buck 1996). Once they emerge, adult reproductives must replenish their lost reserves (Peakin 1972). Males increase their carbohydrate stores and females

Figure 2: Diagram showing the flow of energy-rich nutrients through and annual perennial colonies. The thickness in the arrow depicts the relative amount going to each caste.



increase their lipid stores. Males of the ant, *Pheidole ceres*, increase their carbohydrate stores during their stay in the colony whereas adult females increase their lipid reserves and reduced their carbohydrate reserves (Judd This Volume). The main difference between these two groups is that males are very short-lived whereas females are not. Males need a relatively brief supply of energy {Hölldobler & Wilson, 1990). In Vespid wasps, males are relatively short-lived whereas gynes must survive diapause and then initiate a colony (Reeve 1991). These gynes probably require a substantial amount of fat reserves. It would be interesting to see if the same requirements hold true for females produced by swarm-founding colonies.

If an individual is unable to store enough food on her own, she might need to stay in her natal colony or take enough individuals with her to insure her survival (Keller 1993). This pattern has recently been discovered in the introduced fire ant *Solenopsis invicta.* In this system, there are two types of colonies, monogynous and polygynous. When the queens of monogynous colonies are compared to polygynous queens the monogynous queens are much fatter (DeHeer et al. 1999). They appear to be able to store more reserves. DeHeer et al. (1999) has shown that the thinner, polygynous queens cannot survive on their own and must share their resources with other queens during colony founding. Recently a gene has been uncovered that is involved in controlling how heavy an alate can become (Krieger and Ross 2002). This suggests that the inability to store enough food is a heritable trait and promotes polygyny (Krieger and Ross 2002; Ross and Keller 2002). Whether similar genes exist in queens of other polygynous ants or wasps remains to be seen.

Foraging patterns of the Hymenoptera demonstrate the continuous need for carbohydrates. Bees continuously forage for nectar throughout the year (Michener 1974). Ants accept sugar solutions either at feeders (Judd, This Volume) (Sudd and Sudd 1985; Stein et al. 1990) or from aphids (Sudd and Sudd 1985) throughout the season. In some ants, the preference for the concentration fluctuates but the ants always take some solution (Sudd and Sudd 1985). Colonies of *Pheidole ceres* (Judd This Volume) and *Formica lugubris* (Sudd and Sudd 1985) increase their carbohydrate consumption when adult sexuals are present in colony. Wasps also collect carbohydrates continually. *Polistes* colonies, for example, increase their preference for carbohydrates when the adult sexuals emerge (Reeve 1991). Unlike what has been observed in the flow of nitrogenous nutrients, the entire colony is a sink for energy-rich nutrients.

Food storage

In annual colonies, food storage occurs, but not to any great extent. Several species of wasps are known to have temporary stores of honey in unoccupied cells (Hunt et al. 1998). Of the bees that produce annual colonies, bumblebees stand out as the most advanced food-storers. They build storage vessels out of wax to store nectar and sometimes pollen (Heinrich 1979). However, these reserves only act as safeguards preventing a colony from starving during temporary food shortages.

Long-term food storage appears to have evolved multiple times in social Hymenoptera and appears to coincide with the evolution of perennial colonies. Thus, perennial colonies have the added burden of gathering enough food resources for colonies to survive long-term food shortages such as winter. Honeybee colonies, for example, use

25kg of honey during the winter. This amount of honey is in addition to the 35kg of honey and 20kg of pollen needed during the other seasons (Seeley 1985).

It would be interesting to trace the origin of food storage in both bees and ants as this probably influenced their ability to evolve the perennial colony lifecycle. Since ants have no immediate non-eusocial relatives, this task would be difficult. However, bees cover a large range of sociality and a comparison among bees may provide insight into the origin of food storage. Most bees store food to some degree. Even solitary bees collect pollen and store it with their larvae. This behavior is also found in the subsocial bees. It is possible that in some species, excess food was stored in extra cells and used by the colony members. The storage of extra food is observed in annual bees such as bumblebees (Heinrich 1979). Alternatively, members of these associations could have taken small amounts of food from each other's cells. Colonies that stored extra food would have fared better if a major food shortage occurred. This process could select for better and better food storers and the creation of vessels (cells) specifically for food storage.

Types of food storage

The evolution of the ability to store food required two new mechanisms. First, colonies had to evolve a mechanism to prevent food from spoiling. Second, there had to be a major change in the flow of nutrients through the colony such that a large portion of the nutrient flow was diverted to food stores. This is especially true for colonies found in temperate climates in which the foraging season is limited. The two major groups of perennial Hymenoptera, bees and ants, have developed three distinct food-preserving

strategies. There are two forms of external food storage, termed here as canning and graining, and internal food storage.

Canning: This method of food preservation employed by bees, is akin to pickling techniques used by humans. The food is made inedible to microorganisms and is sealed in a container. Bees store nectar in the form of honey. Honey is a very concentrated solution of glucose, fructose (produced from the hydrolysis of sucrose) and other nutrients found in the nectar gathered (Morse 1972). The concentration of sugars in honey is so high that it acts as a desiccant preventing microorganisms from growing in the stores (Morse 1972). The aforementioned wasp honey has similar properties but it appears to be produced through a different mechanism (Hunt et al. 1998). Bees also store pollen. Both honey and pollen are sealed from the external environment in cells made from wax, adding another layer of protection for the food.

Graining: In this form of storage, seeds are gathered, in some cases dried, and stored in granaries. Dried seeds are another food product that microorganisms find difficult to colonize. In fact, the seed itself is an adaptation to protect the embryo from the natural environment and provide the embryo with all of the energy it needs to sprout (Raven et al. 1999). Seeds tend to be high in both lipids and proteins providing the ants (and humans) with a rich source of energy and nitrogen (Judd This Volume). For an organism trying to store food, the seed is a perfect nutritious, long-term food storage device.

Seed-harvesting has evolved independently several times within Formicidae (Hölldobler and Wilson 1990). These seed-storing ants gather large quantities of seeds, dry them, and then store them under ground. Estimates suggest that the ant

Pogonomyrmex can remove up to 10% of the total seed production of the plant community in one year. The seeds gathered generally comprises of 50% of the seeds of their preferred plant (Whitford 1978).

Why certain seeds are preferred over others has yet to be explored. One possibility is that some seeds are more nutritious than others. Judd (This Volume) found that the preferred seed of *Pheidole ceres*, sagebrush, has a higher percentage of protein per seed than any other seeds it collects. In this case, the ants prefer the most nutritious seeds. However, whether this preference is due to ants cuing in on seed nutritional content remains to be seen.

Internal stores: A third method of food storage used by ants is internal stores. Food can be stored in several places inside the hymenopteran body. Fat bodies, hemolymph, and muscles all have been shown to be potential food storage locales (Wheeler and Martinez 1995; Nation 2002). In some dimorphic ants the majors store a higher percentage of lipids than minors, thus acting as food stores for the colony (Wilson 1974; Stradling 1987; Lachaud et al. 1992; Tschinkel 1993). One could not review internal food stores without mentioning the honey pot ants, *Myrmecocystus mimicus* (Hölldobler and Wilson 1990). These ants have special workers whose abdomens expand well beyond their normal size. These ants just hang in the nest and distribute food to their fellow colony members (Hölldobler and Wilson 1990).

When the three food storage methods are compared, clearly canning takes more time than the other two methods. First, nectar must be broken down into its monomer sugars. Bees have a special enzyme, invertase, which hydrolyzes disaccharides into monosaccharides (Michener 1974). Second, the bees must remove water from the

solution to increase the concentration (Seeley 1997). Finally, the cell must be sealed. Honeybees are probably the best canners in the insect world, having a special caste devoted to this very process. Graining on the other hand takes time but drying seeds does not require constant attention. The food is already packaged; it just needs to be collected. Therefore, the workers are free go about their business and collect more seeds. The main tasks are building the granaries, shuttling the seeds from place to place and finally opening the seeds. Internal food stores are probably the most efficient in terms of time. The only requirement is some internal mechanism allowing for food storage. However, this form of storage is limited by the number of individuals in the colony. Plus, there is an additional cost to carrying around the extra weight. In comparison, canning and seed storage are more advantageous as the potential for storage is limitless.

Reorganization of nutrient flow

The introduction of food storage required an ability to allocate a certain percentage of the incoming resources to storage. Both canners and grainers store products of plant reproduction, pollen, nectar and seeds which coincides with the optimal time for colonies to grow and reproduce. Food-storing Hymenoptera must simultaneously collect food for the colony's immediate needs and food to be saved for future use. The addition of this new task requires that parts of the nutrients collected be shunted to food storage and possibly the introduction of a larger worker caste. As a result, the overall flow of nutrients and information must be organized differently in a colony that stores food verses a colony that does not store food.

Information flow in a Hymenopteran colony

The composition of individuals in a colony changes throughout the year. As a result, the needs of a colony as a whole change as well.. In addition, natural fluctuations in resource availability can affect nutritional needs by colony members. In order to maintain the flow of nutrients into a colony, individuals need a mechanism to determine their colony's needs.

Three types of information impact the flow of nutrients: 1) The nutritional needs of individuals, 2) where the food should be distributed and 3) where the food source is located. Of the three categories, only the first two types of information need to exist for a colony to operate. The third type of information only adds to the efficiency of colony operation.

Information about what to acquire

Foragers are faced with the decision as to what types of foods are needed by the colony. Colonies' needs change, and foragers need a reliable indicator by which to determine the needs of the colony. Foragers can acquire this information from other colony-mates or by assessing their own nutritional state.

Forager-nonforager communication: Since the nutritional fluctuations are caused by the intermittent production of larvae and sexuals, it seems reasonable to predict that larvae may convey their needs to the workers. Worker-larvae communication does seem to occur in annual colonies. *Polistes fuscatus* workers appear to communicate with larvae through the use of nest vibrations (Gamboa and Dew 1981; Downing and Jeanne 1985; Savoyard et al. 1998). Wasp larvae also give food secretions to adults. Thus it is possible

for communication to occur directly through the use of pheromones or indirectly as workers assess the quality of the saliva itself (Hunt 1991).

In large perennial colonies, larvae-forager communication is less likely because the larvae and foragers rarely interact directly. An intermediate caretaker caste usually feeds the larvae. In these colonies, communication between foragers and caretakers would be more likely to be found. In honeybees, the level of pollen foraging is controlled through communication between nurse bees and pollen foragers (Camazine 1993). The nurse bees are the individuals that digest and distribute the protein to the larvae. In addition to this process, these bees also give a small amount of food to pollen foragers. If the food is high in protein or amino acid than the number of pollen foragers is reduced (Camazine 1993, Seeley, 1997). In this case, the larvae are indirectly influencing the workers as they drain the nurse bees' reserves.

There is mixed evidence for larva-worker communication in ants. Brian (1977) found evidence that *Myrmica rubra* larvae influenced the foraging behavior of workers. (Sorenson et al. 1985) showed that *Solenopsis invicta* larvae deprived of lipids increased the colony's intake of oil. In that study however, it appeared that the nurse ants might be involved in this mechanism rather than the larvae. In a different experiment (Cassill and Tschinkel 1995) observed that larvae did not directly influence forager behavior in *S. invicta*. Judd (This Volume) also found that larvae of *Pheidole ceres* do not influence the behavior of the workers. These results are consistent with the hypothesis that larvae of perennial colonies are not interacting with the foragers.

Self-Assessment: Instead of relying on signals from colony-mates, foragers could cue into their own nutritional stores to assess their colony's needs. In a colony, non-foragers are sinks for whatever nutrients they need. Larvae are generally protein sinks whereas reproductive adults are carbohydrate sinks. Thus, the nutrients flow from the gatherers to these sinks. Foragers are the last individuals in a colony to be satiated. They only lose those nutrients that are in demand and as a result have a reliable cue in which to determine what nutrients are needed. However, this process requires that the species utilize internal food stores. Self-assessment probably does not occur in bees because they store all nutrients in excess leaving the forager without a reliable internal cue. Ants do store food internally, so self-assessment is a viable option for foragers.

Several lines of evidence support the hypotheses that ant workers can self-assess. Blanchard et al (2000) found that workers of *Leptothorax albipennis* were more likely to forage when they were low in lipids. Judd (This Volume, Judd This Volume) found that the food preferences of *Pheidole ceres* matched the nutritional states of the ants. When they were low on a particular nutrient they tended to forage for it. Sorenson (1985) reported that foragers preferred nutrients that are lacking in nurse ants. Presumably, the workers themselves were low in these foods. Thus, unlike wasps and bees, ants might use their own state to make foraging decisions rather than rely on information through food exchange. However, the evidence supporting this conclusion is correlative and causation has yet to be directly tested.

Food distribution

Much of this paper discussed where different nutrients go. It is clear from the evidence gathered thus far that nutrients are distributed only where they are needed. This

begs the question of how individuals know where these nutrients should go. One possibility is that caretakers are responding to the needs of their charges. There is little evidence to support this. Cassill and Tschinkle (1995) followed nurse ants during the feeding bouts. They found that the nurse ants appear to deliver equal amounts of food to every larva. In honeybees, most of the incoming food is stored andthe food is later taken as other members need it. Nurse bees eat pollen when they feed larvae, and workers eat honey when needed. In seed harvesting ants, the stored food is separated from the other types of food arriving. It may be that this information is dealt through self-assessment. If a worker is low on a particular nutrient, she replenishes herself through stores or by begging from a returning forager. In this case, the distribution of nutrients is an unconscious result of feedback loops as different nutrients are depleted.

Communication between foragers

Although mechanisms are in place that determine what food types are needed, annual colonies lack a mechanism for workers to recruit others to a food source (Jeanne et al. 1995). Since eusociality evolved multiple times in wasps (Carpenter, 1990), and wasps lack inter-forager communication, inter-forager communication is probably not a criterion for a colony to exist.

However, perennial colonies all show some level of recruitment ability. Ants show communication ranging from tandem running in some smaller ant species (Hölldobler and Wilson 1990), chemical trails (Hölldobler and Wilson 1990) in larger colonies and more permanent trunk trails (Traniello 1989; Salzelemann 1992; Lopez et al. 1994). Bees also have a range of communication mechanisms (Michener 1974). Several stingless bees have dances that alert colony members to food sources (Nieh

1998). The honeybee has the most advanced communication system of all the bees in which a returning forager can tell colony mates the direction, distance, and odor of the food source (von Frisch 1967) These communication processes allow for quick exploitation of a food source (Phillips et al. 1978); Judd This Volume). Ants and social bees are very strong competitors, due partially to the sheer numbers they can quickly send to a food source (Seeley 1987; Traniello 1987; Traniello 1989).

Communication, food storage and life history

It appears that communication between foragers is connected with the evolution of food storage. Perennial colonies have to build food stores at the same time they are growing and reproducing, and their foraging system has to be efficient enough to accomplish this. Sending out large numbers of foragers to scour the landscape is not good enough because only a small percentage of individuals locate a food source and the rest have just wasted time and energy. However, if the foragers are able to communicate the location of a food source to others, than the efficiency of the foraging force increases. The ability to store food and the ability of foragers to communicate with each other was probably essential for the survival of free-living perennial colonies.

This hypothesis that intra-forager communication is necessary for perennial colonies is supported by the fact that wasps, as a group, never developed either long-term storage or communication between foragers (Jeanne et al. 1995), and there really are no true perennial wasp species (Gadagkar 1991; Jeanne 1991). Some of the swarm-founding wasps such as *Polybia* approach the perennial life style (Jeanne 1991), but the colonies do not last long. In many cases, the colonies dissipate into several independent swarms

that abandon the natal nest. All of these swarm-founding wasps are found in the tropics and the pressures that require food storage and communication might be relaxed enough to allow these large wasp colonies to persist.

Bees also offer some evidence for the necessity of inter-forager communication for perennial colonies as well. Food storage exists at some level in all bees (Michener 1974); however, inter-forager communication does not. Bumblebees, as mentioned above, store food but seem not to communicate forager-to-forager. Each forager acts as an independent unit (Heinrich 1979). The lack of communication between foragers probably prevents perennial bumblebee colonies from persisting from year to year because they are unable to gather enough resources to survive. Honeybees on the other hand, have both food storage and inter-forager communication and are able to survive from year to year. Thus, the evolution of intra-forager communication may have allowed bees to make the jump to a perennial lifestyle and made elaborate food storing behaviors more adaptive. Of course, the evidence found in wasps and bees presented here only tentatively support the necessity for inter-forager communication for the evolution of a perennial lifestyle. A phylogenetic analysis of Hymenoptera comparing the two traits would provide a more robust test of this hypothesis.

Conclusions

The nutritional ecology of hymenopteran colonies has much in common with the nutritional ecology of individual organisms. The allocation of nutrients within a colony matches the allocation of these same nutrients within a free-living organism. However, there are many areas to explore. Many nutrients have been virtually left untouched by

students of eusociality. The wealth of research done on individual animals should provide us with testable hypotheses as to the importance, optimal levels, and distribution of these nutrients.

By examining a colony as a mimic of an individual, we can perhaps use colonies to model individuals. Sampling colonies and watching individuals is potentially easier than trying to sample certain tissue from individual animals. Perhaps some of the questions in which using individuals becomes daunting can be easily handled using a colony. This type of comparison may allow us to predict what types of mechanisms to expect in different organisms.

Finally, it is clear that colonies are not always using the same criteria when making decisions. Wasps, bees and ants all have many similarities but several differences stand out. These groups have approached food storage and communication differently. Bees employ inter-worker communication and can their food whereas ants collect seeds and in many cases rely on their own personal stores for cues. Wasps appear not to have evolved either strategy. Perhaps these differences can provide insight into nutritional physiological differences between individuals that survive from year to year and those that live for only a single year.

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Chapter 2: The Nutritional ecology of *Pheidole ceres*: The effects of water and time of year and colony composition on foraging preferences.

Abstract

I examined how changes in environmental conditions and colony composition affect the foraging behavior of the ant, *Pheidole ceres.* Field colonies were presented with a choice between a protein source and a carbohydrate source at three different times in the year under wet and dry conditions. These time periods corresponded with different reproductive (the production of sexuals) and growth (the production of workers) stages of the colony.

Moisture had no effect on the forging behavior of *P. ceres* but the colonies did show a change in foraging behavior during different times of the season. This behavior correlated with the amount of larvae in the colony. However, two lab studies I conducted demonstrated that larvae did not directly influence the foraging decisions of the workers and that adult reproductives did. In the first experiment, larvae fed on carbohydrate or protein diets were placed with workers fed on the opposite diet. In the second, workers were given larvae or adult reproductives to tend and then tested for their food preference. In both experiments, the workers ignored larval needs but foragers tending adult reproductives increased their preference for carbohydrates.

INTRODUCTION

Abiotic conditions, such as temperature and moisture, fluctuate in many natural environments. These changes can impact food availability. Animals adapted to these environmental fluctuations evolved mechanisms that cue in on these changes allowing them to adjust their physiology and behavior in order to maximize reproduction and growth (Morse 1980; Stephens and Krebs 1986). Subsequently, the foraging behavior of these animals varies in accordance with their fluctuating nutritional demands.

In temperate climates, the seasons cycle between warm months, with high food availability, and cold months, with low food availability. Animals adapted to this cycle reproduce during the seasons in which conditions are favorable. Once the less favorable seasons are imminent, these animals switch strategies and store food in order to survive. As animals cycle between reproductive and survival strategies their foraging behavior changes as well (Pohl 1976; Lucas 1989). This pattern has been well documented in solitary animals. When birds (Levey and Stiles 1992) and mammals (Davis 1976; Hill and Florant 1997) reproduce, they increase their protein intake, in order to feed growing young. These same animals switch to high-energy compounds such as carbohydrates and lipids when storing food for winter.

In addition to the long-term seasonal changes, short-term less predictable changes can also occur. In arid climates, moisture potentially affects foraging choices. Kangaroo rats, for example, prefer seeds high in lipids to those high in protein in a normal dry environment (Frank 1988). However, when the same choice is presented in a humid environment the kangaroo rat chooses the seeds high in protein. The kangaroo rat's decisions are driven by the fact that protein digestion costs metabolic water and lipid

digestion produces metabolic water. Thus, a simple rainstorm could alter the foraging behavior of the animal (Frank 1988).

Eusocial insect colonies that inhabit temperate zones face the same issues as solitary animals. However, in a colony several independently acting individuals have to somehow make similar decisions so that the colony as a whole behaves like a solitary individual. There have been few studies documenting these changes. *Polistes* workers forage for protein rich caterpillars when the larvae are in the colony and switch to nectar once the reproductive adults emerge (West-Eberhard 1969; Hoshikawa 1981; Tsuchida 1991; O'Donnell 1998). However, only the gynes of *Polistes,* rather then the colony as a whole, survive to the following year (Reeve 1991). Stein et al (1990) showed a population of the imported fire ant, *Solenopsis invicta,* changes its diet, foraging for protein during warmer months when the colonies are reproducing and for carbohydrates during colder months when the colony is in its growth stage (Oster and Wilson 1978). However, the imported fire ant inhabits areas in which the winter is not cold enough to completely stop the colony from foraging. A better test of the effects of seasonal changes is to examine a eusocial animal that over-winters as a colony and lives in a climate in which the winters are harsh enough to prevent foraging.

Since the colony's needs are changing, mechanisms should be in place to allow foragers to adjust their behavior to match the needs of other colony members. One possible mechanism is the other colony members communicate their needs to the workers and the workers adjust their foraging behavior accordingly. In honeybees, the likelihood of a returning forager to recruit other foragers or nectar receivers depends on the length of time it takes for her to find a nectar receiver to unload her (Seeley 1993; Seeley 1997).

Polistes fuscatus larval responses to nest vibrations produced by adults allow the adults to ascertain the nutritional needs of the larvae (Savoyard et al. 1998). Thus, it is possible for a worker's behavior to be influenced by fellow workers as well as non-workers.

In ants, the evidence that larvae influence workers' foraging behavior is mixed at best. Brian and Abbot (1977) found evidence that larvae of *Myrmica rubra* influenced the types of foods workers foraged for. In the army ant, *Neivamrymex nigrescens*, larval hunger will increase the likelihood a colony will emigrate (Topoff and Mirenda 1980). Sorenson et al. (1985)} found that the presence of starved larvae in *Solenopsis invicta* colonies increased the amount of lipids (oil) brought back to the colony. However, Cassil and Tschinkle (1995) found that larvae of *S. invicta* seemed to have no effect on foraging biases of adult workers. In the latter two cases, these conclusions are based on correlative evidence rather then a direct experiment, possibly explaining the conflicting results.

In this study, I examined how external cues such as season and water and the internal cues such as the colony composition affect foraging behavior of the ant *Pheidole ceres* over the course of the year. *P. ceres* is a small ant found in a mountainous climate that is very arid and the winters are harsh. This provides a natural situation in which water stress, an immediate problem, is coupled with longer-term climate change. As with all ants in this environment, colonies of *P. ceres* stop foraging during the winter (Personal observation) so they have to store enough food to survive and produce new reproductives when conditions are favorable.

I presented field colonies of *P. ceres* with a choice between proteins and carbohydrates at different times of the year. The same choice was tested under both wet and dry conditions within each time-period. This allowed me to look for seasonal and

short-term effects on *P. ceres*'s foraging behavior. The ants' foraging choices were also compared with the colony composition at each time interval.

In addition to field trials, laboratory colonies of *P. ceres* were given a choice between protein and carbohydrates under one of two possible conditions: 1) colonies which contained only larvae or adult reproductives and 2) colonies in which larvae were deprived of one type of nutrient and workers of a different type of nutrient. Both experiments tested whether non-workers influence worker foraging behavior.

METHODS

General Methods:

Study site: My study site was located in the Rocky Mountains (Larimer County, Colorado) at about 7,500 ft above sea level. The ant colonies were located on a south facing slope covered with patchy vegetation (predominantly small shrubs, cacti, and grass). The slope received a lot of direct sunlight. Owing to the heat, very few ant species foraged during the day.

Study Organisms: *Pheidole ceres* (Gregg 1963) colonies are typically found under flat rocks in open areas. Nests of *P. ceres* tend to have multiple entrances, most of which emerge from the rock or nearby. Multiple entrances enable the colony to exploit multiple food sources simultaneously. Foragers are most active during evenings, mornings, and at night when the temperature is above 10°C. Foragers show a strong aversion to direct sunlight.

Colonies of *P. ceres* are monogynous. There are 700-1000 individuals in a typical colony. As with all species in the genus *Pheidole,* there are two distinct worker castes, majors and minors: 88.5% (± 15%) are minors. Minors and majors forage at the same food sources but only minors appear able to recruit more foragers. Despite their name, *P. ceres* are generalist feeders. They eat seeds, dead insects and even milk aphids for honeydew (personal observation).

Field Experiments

Surveys: I collected baseline data on ten colonies once a month from April 2000 until October 2000. During the surveys, I categorized the number of larvae, pupae of both workers and sexuals as, absent, few, some, many. This circumvented destructive sampling. I also noted the presence or absence of adult males and female sexuals.

Feeders: I created feeders that allowed me to control the food sources and to easily assess the numbers of recruits. Each feeder consisted of a small round (6 cm) disk cut from translucent red plastic disk with eight grooves (about 2cm long) evenly spaced radiating from the center. A small PVC pipe cap (2.5 cm in diameter) was inverted onto the disk so that the liquid placed in the cap flowed into the troughs (the ants appeared to be able to get to the liquid in the cap from any point where the cap rested on the disc). A vacuum created in the pipe cap prevented overflow. This feeder was similar to the bee feeders described by von Frisch (1967). To prevent the *P. ceres* foragers from being disturbed while they foraged, I lit the feeders from underneath with a 1.5 V bulb placed in an inverted petri dish of the same diameter as the feeder and buried under the soil. Any

light from above such as a flashlight beam disturbed the ants, even if it was filtered for red wavelengths. However, when the feeder was lit from underneath, the ants foraged readily on it.

Trials: Two feeders were both placed equidistant (30cm) from the colony. One feeder contained 20% sucrose solution with 50 µl/l solution of anise extract (to attract the initial scouts to the feeder: see Seeley, 1991; anise has no effect on the preference in *P. ceres* (personal observation) as recruits are following chemical trails not the food scent). The other contained 20% egg white solution (egg white has a fairly strong smell so a scent was not necessary). The two feeders were placed on the left and right of the colony at random (flipping a coin) in the clear patches of soil such that there were no clumps of vegetation between the feeder and colony entrance.

Once the ants discovered one of the two feeders, I recorded the number of ants on each feeder every five minutes until one hour after the second feeder was discovered. If one feeder was not located in 40 minutes the trial was ended to prevent any satiation effects.

The effects of time of year and soil moisture on protein vs. carbohydrate preference: Colonies were tested during three time periods, representing different events during the colony cycle. The three periods were as follows:

Period 1: Spring: May- both worker and reproductive larvae present

Period 2: Mid Summer: July- Adult reproductives and worker larvae present

Period 3: Late Summer: Early September- Early October- Worker larvae only.

Within each time period ten colonies were tested for their preference between carbohydrates and proteins under wet soil and dry soil conditions. All trials were conducted no less then two days after a day long rain.

The wet treatment consisted of sprinkling 10 liters of water on the colony and the surrounding soil (about a 50 cm radius) two hours before a trial. This simulated a heavy rainfall. The dry treatment was a control.

Ten colonies were randomly divided into two arbitrary groups (Group 1 and Group 2). A colony from Group 1 was paired with a colony from Group 2. The experiment was divided into two rounds. In the first round, the colonies within Group 2 were given the wet treatment and colonies from Group 1 were kept dry. A pair of colonies was tested for protein vs. carbohydrate preference on the same day. Once all the colonies were tested, I reversed the treatment in round 2 so that colonies from Group 1 received the wet treatment, and colonies within Group 2 received the dry treatment. In this fashion all the colonies received both treatments, and both a wet and a dry colony were tested on the same day. All colonies were tested at roughly the same time of the evening.

Data Analysis: The number of foragers at the feeder leveled out about 30 minutes into the trials. For each trial, I took the last four measurements and used the mean of those measurements to get a final score for the foraging preferences of each colony. For intra-colony comparisons, these measurements were converted to the percent of foragers at the carbohydrate feeder. This conversion controlled for colony size. This was done for all ten colonies in all three foraging periods. Majors and minors were analyzed

separately. Unless otherwise noted, I used the Friedman's Test followed by a Multiple Comparisons Procedure (MCP) for the analyses.

Lab Experiments:

Collections: Ten mature colonies were dug up in the field and transported back to the lab. The colonies were then sorted from the soil and moved into the experimental setup described below.

Nestboxes: Nestboxes were made from Gladware® plastic salad containers (709 ml). The boxes were half-filled with dental plaster. A square indentation in the middle of the plaster with a plastic cover provided the actual living quarters for the colony. Two holes were drilled through the opposite sides of the container into the nest cavity so that the ants could readily go in and out of the nestbox.

Arenas: The occupied nestboxes were kept in arenas made from Sterilite® 11.4 L boxes (42cm x 29.5cm x 16.5cm). A layer of plaster was placed on the arena's bottom to control the moisture levels. A small indentation was created in the arena for the nestbox. The walls of the arena were covered with Fluon® to prevent escape. In order for the ants to forage normally, a light layer of dirt was placed in the arena.

Trials: Two feeders were placed in opposite corners of the arena. One feeder contained 10% egg white solution (protein) and the other 10% sucrose solution (carbohydrate). Once a feeder was discovered the number of recruits at both feeders was counted every 5 minutes for 1 hour. The second feeder was generally discovered within 5 minutes of the first. Three experiments were performed using this basic setup.

Experiment I: Brood switching experiment.

Ten colonies were divided into four equal subunits and placed in the experimental arenas described above. Two of the units (A and B; Figure 1) were fed carbohydrates (10% sucrose solution) *ad lib* and the other two (C and D; Figure 1) protein (10% egg white solution) *ad lib*. The subunits were left alone for 5 days in a room with 12/12hr light/dark cycle. After the 5 day period, the subunits were tested for their food preference. After the trial, the subunits were put back on their diets for another 5 days. After day 10, the brood from unit B (carbohydrate fed) and the brood for unit C (protein fed) were switched (Figure 1) between the two subunits. To control for disturbance, the larvae from subunit A and subunit D were removed and placed back into the same subunit. In all cases the larvae were quickly moved into the nestbox by the ants. After the larvae were switched, I had subunits with the following conditions for each of the ten colonies.

A. Protein deprived workers and carbohydrate deprived larvae

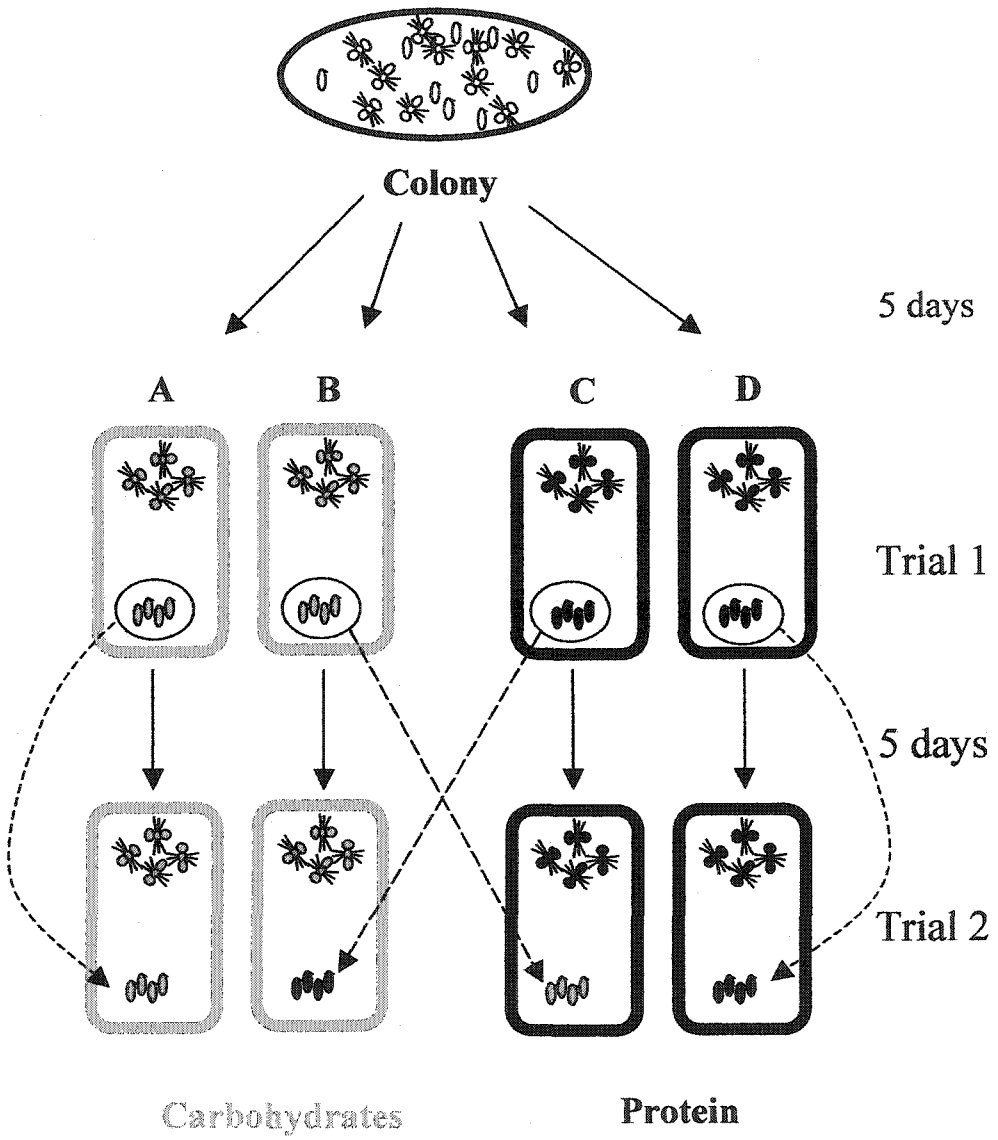
B. Carbohydrate deprived workers and protein deprived larvae

C. Protein deprived workers and larvae

D. Carbohydrate deprived workers and larvae.

The colonies were allowed to sit for at least four hours after the larvae were moved and then I did the second feeding trial. Use of the colonies was staggered across the week such that the experiment was initiated for only two colonies each night. This allowed me to test each colony at the same time each night. At the end of the final trial,

Figure 1. The brood switching experiment. A single colony was divided into four subunits (A-D). Each subunit was given either carbohydrates (gray) or proteins (black). Trial 1 was performed without larval switching. Trial 2 was done after the larvae were switched between subunits B and C. The larvae were removed and then returned in subunits A and D to control for disturbance.



numbers of larvae were counted again to insure that larval death did not influence the results.

This experiment was done in August 2000 and repeated in May 2001. Testing the colonies in both May and August controlled for any affect that seasonality might have had on the worker-larvae interactions

Experiment II: Splitting Experiment.

In this experiment, I tested the ability of the brood, adult sexuals and majors to affect the food preference of minors. Ten colonies were collected in late July 2001 when both larvae and adult sexuals were present. Each colony was subdivided into four subunits that contained the following:

A. 200 minors and all of the adult sexuals

B. 200 minors and all of the brood (larvae and pupae)

C. 200 minors (control)

D. 200 minors and all of the majors

The colonies were kept in a 12/12 light dark cycle for three days without food. During the night after the last day, I tested the colonies for their food preference. This experiment was staggered across the week in the same manor as experiment 1.

Experiment III: Wet/Dry Experiment.

This experiment tested the effects of humidity of the foraging behavior of *Pheidole ceres.* Ten colonies were divided into two subunits, humid and dry.

The arenas and nestboxes used for the humid subunits were soaked in water overnight prior to adding ants. This insured the plaster was saturated with water. After the bin was soaked, a small layer of dirt was placed in the arena. Once the ants were added, the arena was sealed using clear plastic wrap and tape.

The arenas and nestboxes used for the dry subunits had their moisture removed by placing a petri dish containing Drierite® in them overnight prior to adding ants. Once the ants were added, the petri dish with Drierite® was placed in the arena on top of the nestbox to prevent any humidity from building up in the box. A screen prevented an ant from getting into the dish. The arena was then covered with clear plastic wrap and tape to maintain the low humidity.

I let the colonies stay in 12/12 light dark room for 48 hours without food. I didn't let the colonies sit more than 48 hours to prevent larvae in the dry bin from desiccating. After 48 hours elapsed, the humidity within each subunit's arena was measured using a VWR Traceable® hygrometer/thermometer. Each subunit was tested for their food preference. Colonies were staggered across a week as in experiments 1 and 2.

Data analysis:

I analyzed the data from the laboratory experiments in a similar manner as the field experiments. In these analyses, I used the numbers of ants instead of percent ants because all of the subunits were the same size. There was no need to adjust for colony size. Unless otherwise noted, I used the Friedman's Test followed by a Multiple Comparisons Procedure (MCP) for the analyses.

RESULTS

Field Experiments

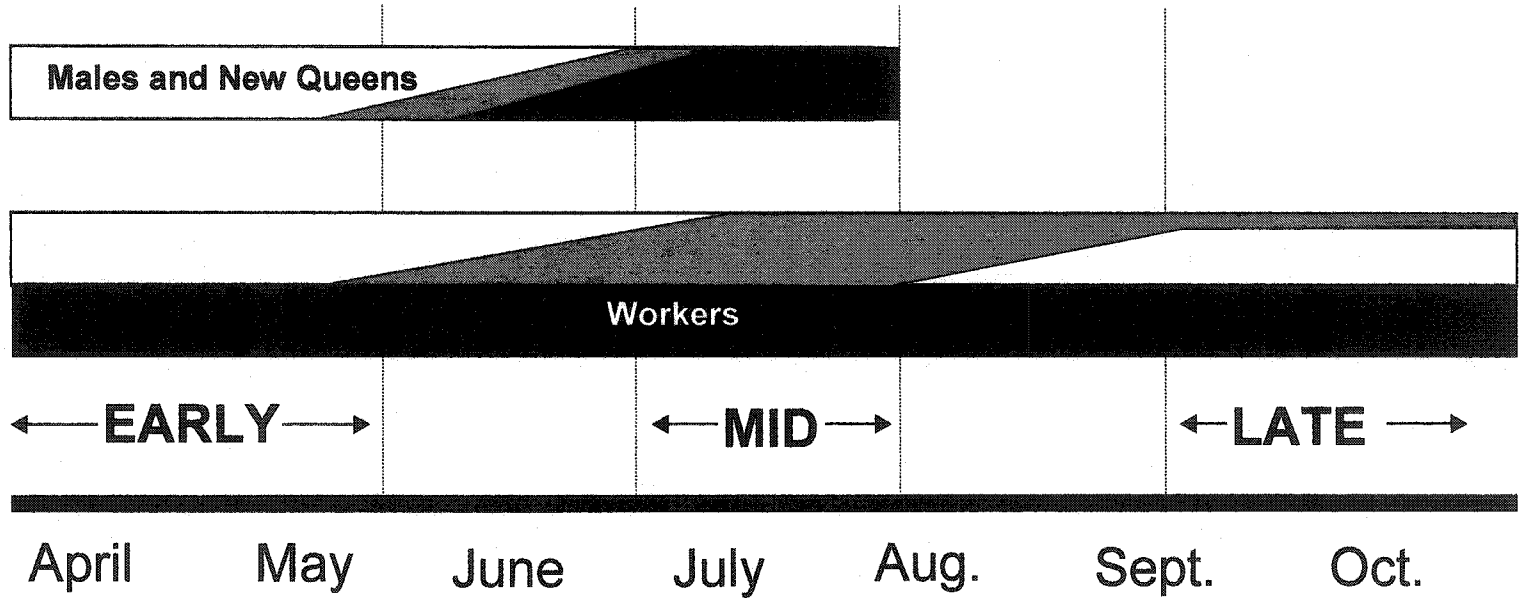
Surveys:

The colonies of *P. ceres* I surveyed were synchronized throughout the year. The colony composition of a typical *P. ceres* colony is shown in Figure 2. During the spring (April – May), the brood consisted of mostly worker (minors and majors) larvae, and reproductive larvae. These larvae appeared to pupate during the early summer (June), and at this time there were very few larvae present in the colonies. The reproductive adults emerged during mid summer (Early July) and remained in the nest for about a month. During this time there were many eggs and pupae but very few larvae. The reproductives departed at the beginning of August. From that point on, there were both larvae and pupae present for the rest of the ants' active season (until late Oct).

Foraging Experiments

Behavior of foragers: The recruitment behavior of *Pheidole ceres* was similar to that found in *Pheidole morrisi* (Johnson 1988). A single minor (scout) found the food source and fed for approximately 3 minutes, usually stopping midway for a few seconds. The scout then headed back to the colony. Less then 8 minutes later a stream of foragers, usually a few majors and many minors, headed to the feeder from a colony entrance. Once the recruitment to the feeder began, continuous streams of workers left and arrived from it. Scouts showed the same recruitment behavior for both protein and carbohydrate sources.

Figure 2. The change in colony composition of a typical colony of *Pheidole ceres* over the course of the year.The relative amounts of adults (black), pupae (stripe) and larvae (white) are shown for both reproductives (males and females) and workers (minors and majors).



The two foraging trails emerged from two separate entrances from the colony. These entrances usually appeared near the edge of the same rock, indicating they are from the same colony.

Numbers of Foragers: The absolute numbers of foragers were compared for the ten colonies in all three periods (Figure 3). There was no significant difference in the total number of minors (N=10, p= 0.68, Friedman's Test) or majors (N=10, p= 0.39, Friedman's Test) outside each colony in all three periods. Thus, each colony was capable of sending out the same number of recruits for each time period.

Effects of water: There were no significant effects from adding water to the soil around the colonies in either the early or the Mid period (Figure 4). This was true for both minors (Early: N=10 p=0.575; Mid: N=9 p=0.260, Wilcoxon Matched Pairs Test) and majors (Early: N=10 p=0.374; Mid: N=9 p=0.999, Wilcoxon Matched Pairs Test). The late period was not tested for the effects of water, because an early snowfall prevented completion of the latter half of the trial. Results from the early and mid period suggested there was no significant effect during this time period either.

Effects of time of year: There was a remarkable change between the foraging choice of minors and major in the different time periods. Figure 5 shows the foraging patterns of a single colony, P23, in all three periods. In the Early period there were equal numbers of foragers at the carbohydrate and protein feeder. In the Mid period the colony showed an increase in carbohydrate intake and a decrease in protein intake. The Late

Figure 3. The medians and quartiles of the absolute number of A) minors and B) majors for all ten *Pheidole ceres* colonies. There were no significant differences in the absolute numbers of minors or majors in all three periods (Friedman's Test).

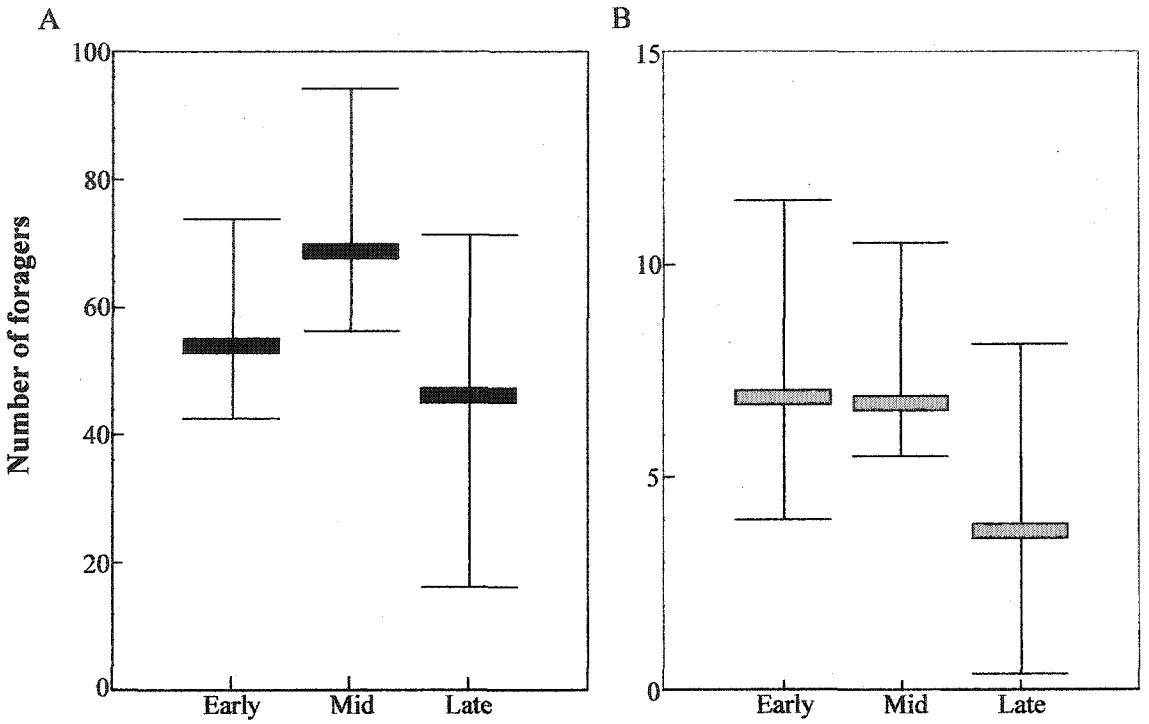


Figure 4. The medians and quartiles of the percent number of *Pheidole ceres* minors and majors at the carbohydrate feeders during the wet and dry treatments. There were no significant differences between wet and dry trials.

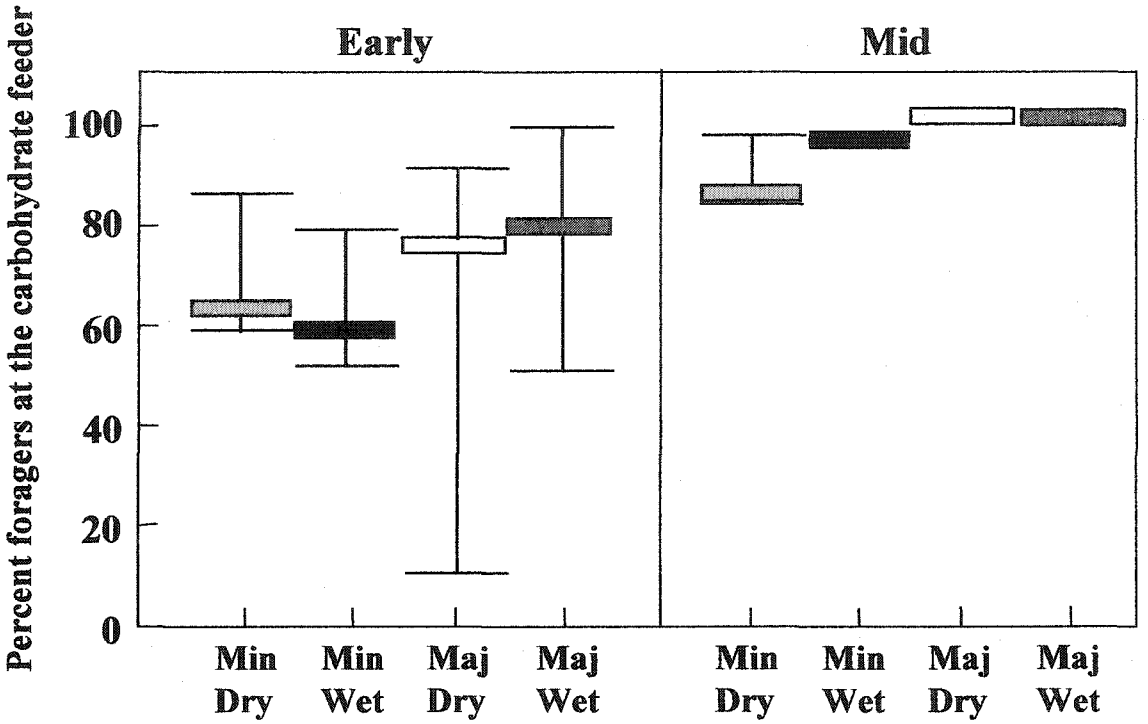
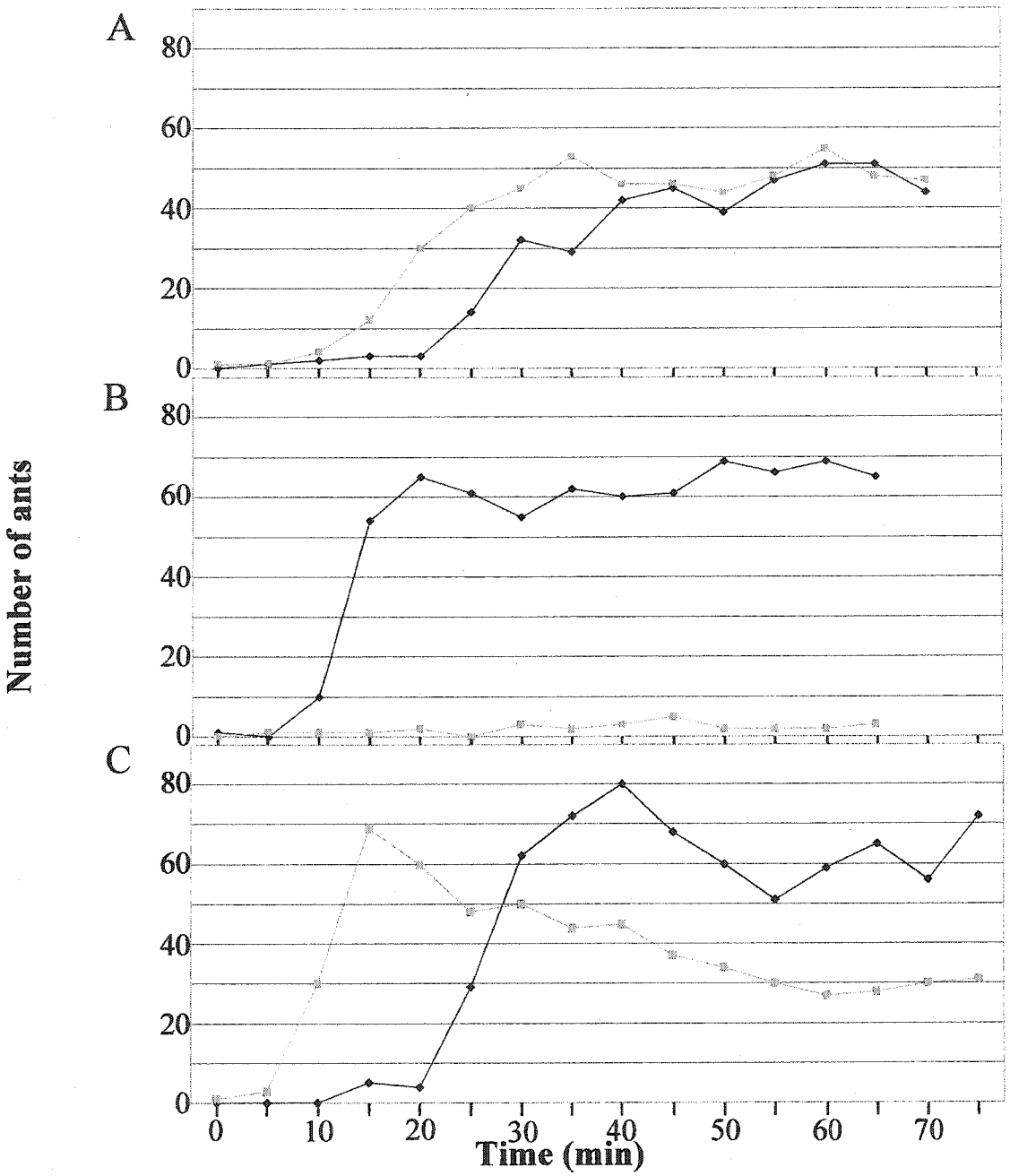


Figure 5. The foraging preferences between a protein feeder (gray) and a carbohydrate feeder (black) for a single *Pheidole ceres* colony (P23) during the A) Early period, B) Mid Period and C) Late period.



period showed an increase in the number of minors at the protein feeder but no change in the majors.

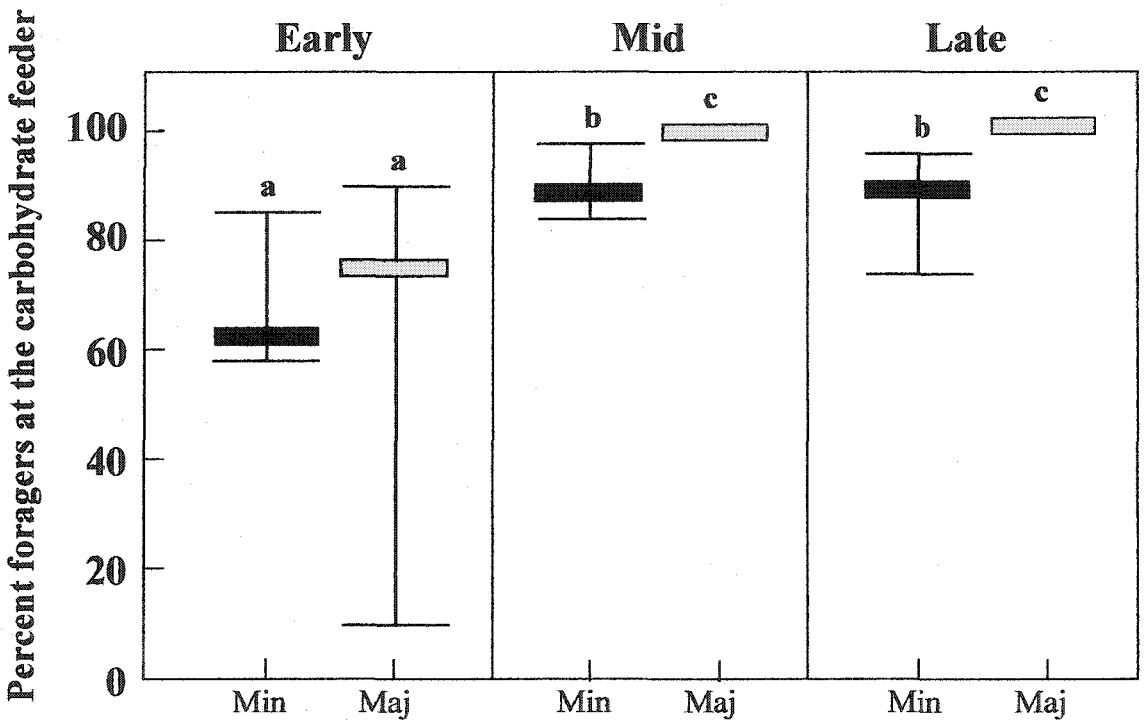
In all ten colonies, minors showed a significant change in foraging decisions across the three periods (N=8, p= 0.030, Friedman's Test; Figure 6). There was a significant increase from the Early Period to the Mid period (MCP). The minors showed a non-significant drop in preference for carbohydrates from the Mid period to the Late period, (MCP).

The majors of all ten colonies showed an even more dramatic change from the Early period to the Mid period (N=6, p= 0.0045, Friedman's Test; MCP; Figure 6). In fact 100% of the majors went to the carbohydrate feeder in the Mid period. This food choice was maintained in the Late period.

Food Preference: Food preference for both minors and majors during all three periods was tested using a Friedman's Test followed by a Multiple Comparisons Procedure (MCP) (Figure 6). Minors showed no significant preference during the Early period. During both the Mid and Late periods the minors had a highly significant preference for carbohydrates (p<0.00001Friedmans Test; MCP). Majors showed a significant preference for carbohydrates (P=0.0001; Friedman's Test, MCP) in the Mid period. However, majors showed no preference in either the Early and Late periods (MCP).

Majors vs. minors: Majors and minors were compared for each time period (Figure 6) using the Wilcoxon Matched Pairs Test followed by a Bonferoni Table wide

Figure 6. The medians and quartiles depicting the foraging preferences of all the colonies between a protein feeder and carbohydrate feeder during the three time periods. The minors (black) and majors (gray) showed a significant change in preference over the three time periods. The letters summarize the significant differences for within caste comparisons (between time periods; Friedman's test and Multiple Comparison Procedure) and between caste comparisons (within time periods; Wilcoxon Matched Pairs Test).



correction (Rice 1989). Majors and minors showed no significant differences in foraging choices in the Early period (N=10 p= 0.65). However, majors showed a significantly higher preference for carbohydrates in both the Mid and Late period (N=9 p= 0.0077; N=6 p=0.028). This suggests that majors and minors are not making the same foraging decisions.

Lab Experiements:

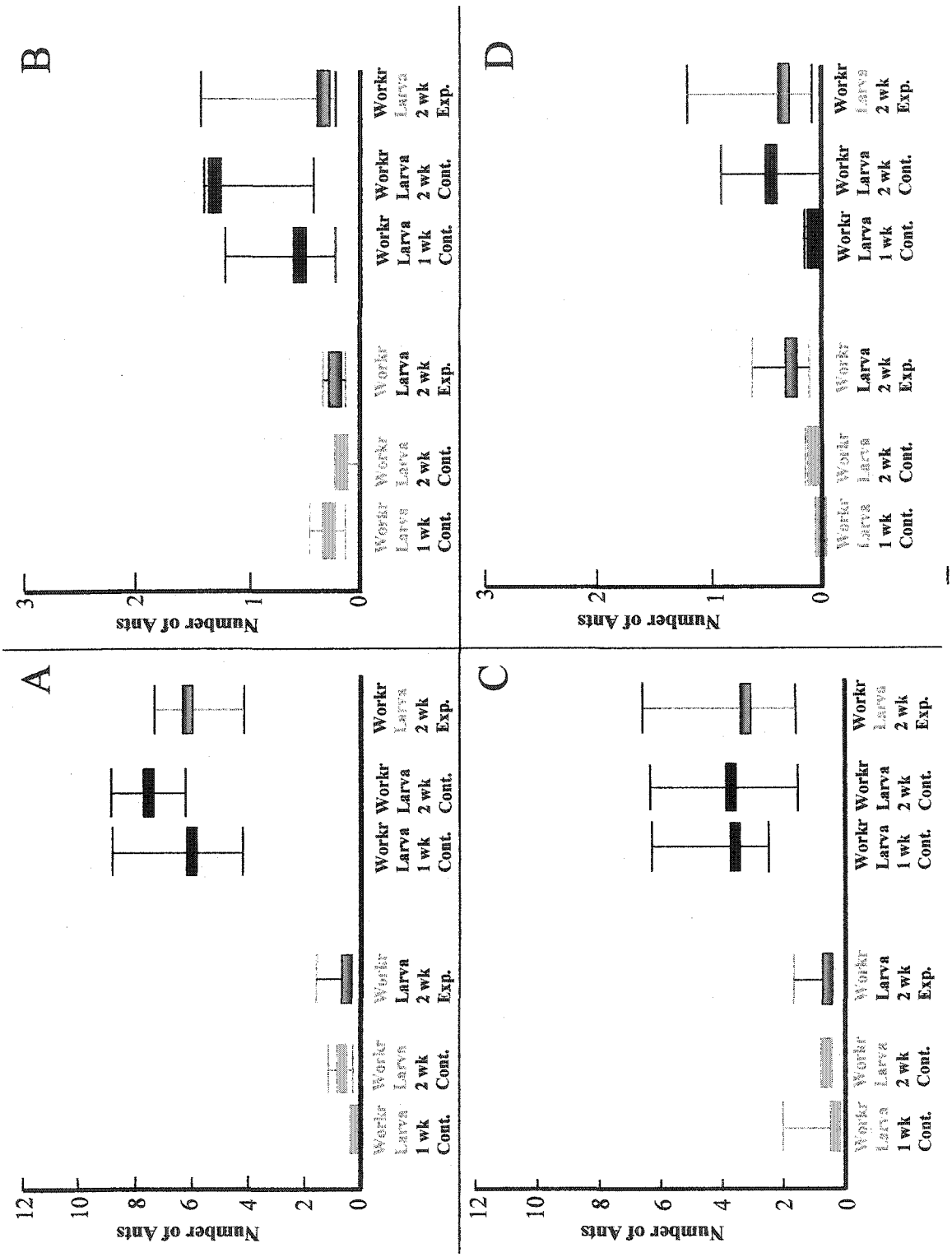
Experiment I: Brood switching experiment

In the following description of the results, I use the following notation that corresponds to Figure 1. The Letters A-D refer to the subunit and the number 1 or 2 to refer to the trial. Thus, B2 refers to subunit B in trial 2.

Results of August 2000

In the brood switching experiment, I compared the preference of the larvae-switched subunit's 2nd trial with its results from the 1st trial (B2 & B1 and C2 & C1; Figure 7A&B), the control, and the other larvae-switched subunit's 2nd trial (B2 & C2: Figure 7A&B). The protein fed subunit foraged significantly more for carbohydrates than the carbohydrate fed subunit (Wilcoxin signed ranked test: B2& C2; p= 0.0069, T=1 Z=2.7; Figure 7A). Each brood-switched subunit showed the same food preference as the control and their previous trial (Carbohydrate B1& B2; p= 0.041, T=7.5, z= 2.04 B2& A2; p= 0.91 T= 21, z= 0.12: Figure 7A Protein: C1 & C2; p= 0.72, T= 24, z = .36, C2 & D2; p= 0.11, T=9, z= 1.6:Figure 7B). Thus, brood switching had no effect on foraging behavior of workers.

Figure 7. Comparisons of the medians and quartiles of the protein fed subunits and carbohydrate fed subunits from the brood switching experiment for both August 2000 (A: Carbohydrate feeder & B: Protein feeder) and May 2001 (C: Carbohydrate feeder & D: Protein feeder). In each graph the two controls (from week 1 and week 2) and the experimental group (received larvae from the subgroup of the opposite diet) are shown for both the carbohydrate fed subunits and protein fed subunits. The gray indicates carbohydrate fed individuals and the black protein fed individuals.



Results of May 2001

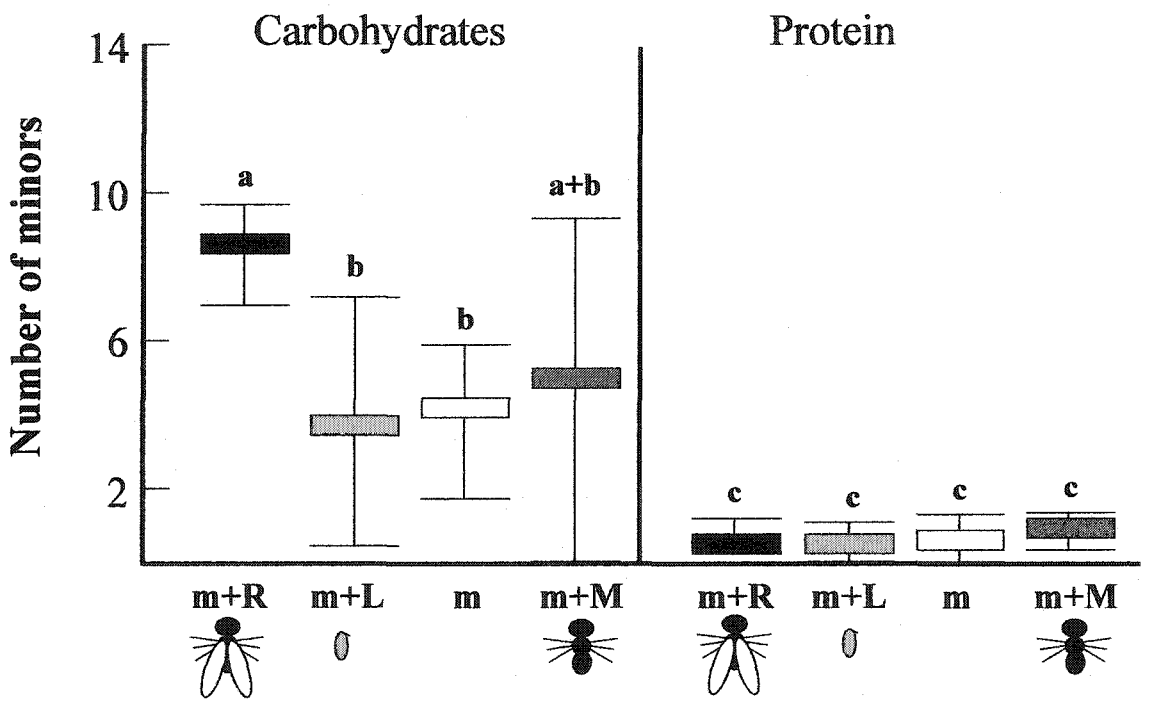
I compared the subunits in the same manner as the August 2000 experiment. The results were exactly the same as seen in August 2000 for both the comparisons between the protein vs. carbohydrate subunits (Wilcoxin signed ranked test: B2& C2; $p= 0.0069$, $T=1$ $Z=2.7$: Figure 7C&D), and the brood-switched subunits and control subunits (Carbohydrate B1& B2; $p= 0.89$, $T=17$, $z= 0.14$ B2& A2; $p= 0.96$ $T= 27$, $z= 0.05$: Figure 7C Protein: C1 & C2; $p= 0.51$, $T= 17$, $z = .65$, C2 & D2; $p= 0.88$, $T=27$, $z= 0.15$: Figure 7D). Thus, time of year had no effect on these results.

Although larval mortality was high (80% ±2%), there was no difference in mortality between the carbohydrate and protein fed subunits. Thus, larvae were probably not being sacrificed in the protein-deprived subunits.

Experiment II: Splitting experiment.

The reproductive subunits showed higher recruitment to carbohydrates than did the control subunits or the brood subunits (Friedman's Test & Multiple Comparisons Procedure: $p=0.056$, $X^2 = 7.56$, $N=10$: Figure 8). The subunit containing majors showed no significant differences from all three subunits (Multiple Comparisons Procedure) probably due to the considerable variation. There were no significant differences in the recruitment of workers to the protein source in four subunits (Friedman's Test: $p= 0.31$, $X^2= 3.53$, $N=10$: Figure 8). This suggests that the reproductives can increase the amount of carbohydrates taken in by the colonies but the larvae have no effect on the behavior of the foragers.

Figure 8. The comparisons of the medians and quartiles of the four subunitss (m+R: minors and reproductives m+L: minors and larvae m: just minors m+M: Minors and majors) of all ten colonies in the splitting experiment for both the carbohydrate feeders and the protein feeders. Each feeder type was analyzed separately. The different letters indicate significant differences based on the Friedman's test p=0.05 and the Multiple Comparisons Procedure.

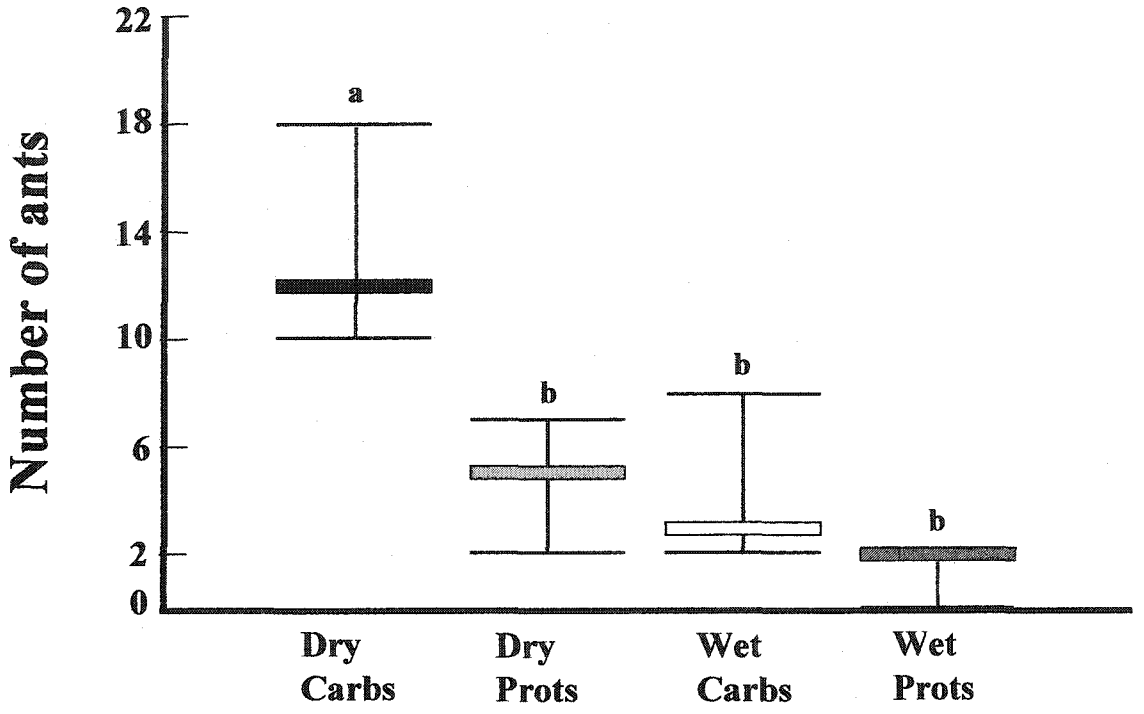


Experiment III: Wet/Dry Experiment

The Dry bins had a mean humidity of 38.28 +_ 9.04 RH% (Range: 23.0 – 50.0 RH%) and the Wet bins had a mean humidity of 95.7 +- 1.18 RH% (Range: 94.4 – 98.2 RH%).

The colonies showed a significantly higher recruitment to carbohydrates (Wilcoxin Signed Ranked Test: P= 0.0077, T=0, Z= 2.67) but not to proteins (Wilcoxin Signed Ranked Test: P= 0.17, T=6, Z= 1.35) in the dry subunits then the humid subunits (Figure 9). There was a significant preference for carbohydrates in the dry subunits (Wilcoxin Signed Ranked Test: P= 0.012, T=0, Z= 2.52) but not in the humid subunits (Wilcoxin Signed Ranked Test: P= 0.29, T=10.5, Z= 1.05). This difference could be explained by the lower numbers of individuals foraging for carbohydrates in the humid subunits (Figure 9) and not by an increase in forager numbers at protein feeders in the wet subunits.

Figure 9. Comparisons of the medians and quartiles of the wet/dry experiment. There was a significantly larger amount of ants foraging for carbohydrates in the dry subunit then all of the other three feeders.



Discussion

The results show that colonies of *Pheidole ceres* responded to seasonal changes but not to the simulated weather effects. There was no change in feeding preferences under dry and wet conditions.

Moisture effects

The absence of an effect of water in both the lab and field experiments suggests that small changes, such as rainstorms, have no effect on the foraging preferences of *P. ceres.* Although insects living in arid climates are generally concerned with water loss, metabolic water loss may not be an important component (Edney 1977). Most of the water loss experienced by arthropods is through respiration and excretion (Edney 1977) or loss of the protective wax coating due to digging in soil [Johnson, 2000 #6945]. Water loss due to respiration did affect foraging decisions of *Messor pergandei* (Feener and Lighton 1991). In this species, water content affected the load size and distance traveled by workers. In my study, the distance to both feeders was the same so the amount of water loss due to respiration was equal for ants attending either feeder. Unlike *M. pergandei, P. ceres* foraged at night, which may help to reduce water loss caused by heat from solar radiation (Edney 1977). Foragers appeared to be uninvolved in colony-level water regulation.

There are other ways for a colony to regulate moisture levels. Most ground dwelling ant colonies are able to adjust their moisture levels through nest architecture (Hölldobler and Wilson 1990). Scherba (1959) found that nests of *Formica ulkei* were maintained at 30% humidity. He demonstrated that the larvae of *F. ulkei,* which consume

most of the protein collected, were moved to moisture levels ideal for digesting proteins. These colony level adaptations would prevent the need for foragers to adjust their foraging decisions in an arid environment.

Seasonal Effects

There was a strong seasonal effect on food preferences in *P. ceres*. In the spring, colonies showed no preference for proteins vs. carbohydrates. In the summer, the ants changed their behavior and showed a strong preference for carbohydrates. This preference for carbohydrates was maintained into the fall although it may have dropped slightly.

This food preference matched the types of individuals being reared. The highest preference for protein occurred during the spring when large numbers of larvae were present. Larvae are normally the chief consumers of protein in a colony (Vinson 1968; Stradling 1987) and their presence would drive up the need for protein. When the reproductive adults were present (Mid Period), the colony increased its intake of carbohydrates. The reproductives needed to acquire energy rich foods to fatten up for their mating flight and to start a new colony (Hölldobler and Wilson 1990). Although some protein has been shown to be stored at this time by adult sexuals (Wheeler and Buck 1992; Wheeler and Martinez 1995), it doesn't appear to affect the foraging decisions of the ants. Workers may have accumulated protein prior to the emergence of the reproductive adults. At the time the adult reproductives were present, there were small numbers of larvae in the colony. During this period, ants dropped their intake of protein and increase their intake of carbohydrates.

A similar pattern was seen in *Solenopsis invicta*. Colonies were shown to increase their intake of protein during warmer months when larvae are generally found in the nest. During colder months, larvae were not feeding and the colonies collected more carbohydrates (Stein et al. 1990). In Stein et al.'s study and this study, the foragers appear to be adjusting their behavior according to the nutritional needs of the colony.

In the fall, the colonies of *P. ceres* retained their preference for carbohydrates despite the presence of larvae. In this case, the colonies may be switching from reproductive strategies to survival strategies. Unlike *Solenopsis invicta*, *Pheidole ceres* is unable to forage during the winter months, and must gather enough resources in order for the colony to survive the winter. Food storage can be accomplished in two ways, by seed collection and physically storing food in their bodies. *P. ceres* gathers seeds throughout the year. The colonies presumably store them in underground chambers much like other seed gathering ants (Wheeler and Rissing 1975; Hölldobler and Wilson 1990). In addition to seeds, individual ants could store food inside their bodies. Many insects store lipids inside the fat bodies. Several studies (Wilson 1974; Lachaud et al. 1992; Tschinkel 1993) found that larger workers store proportionally more lipids than minors. In this study, the majors showed a significantly higher preference for carbohydrates than the minors during the last two trial periods. It is possible that the majors of *P. ceres* are acting as fat stores in the same manner as the majors of *S. invicta*. Majors rarely take on these responsibilities such as feeding larvae or the queen in natural colonies (Wilson 1984; Wilson 1985; Brown and Traniello 1998), and thus may be able initiate food stores earlier than the minors who are feeding other colony members. Although the majors are about 10% of the worker population, they are much larger than minors and could possibly

store more food. Somehow, these individuals change their foraging strategy from gathering nutrients for reproduction to gathering nutrients for storage. The seasonal shift is clear in *P. ceres* but what drives this shift is unclear.

The effects of colony composition

The results clearly show that larval hunger does not affect the foraging behavior of the workers, which matches the findings of Cassil and Tshinkle (1995) for *Solenopsis invicta*. However, the presence of adult reproductives causes workers to forage for more carbohydrates.

Why are the adult reproductives but not the larvae able to influence worker foraging behavior? On a proximate level, this influence might be due to the mobility of the individual in question. Larvae are immobile and depend on the nurse ants. It is the nurse ants that actually interact with the foragers, not the larvae themselves (Sorenson et al. 1985). The reproductives, on the other hand, are mobile and can interact with foragers directly. Adult reproductives are possibly intercepting returning foragers before another worker unloads them. During many lab and field experiments, I saw reproductives come out to artificial food sources. Although the reproductives did not feed from the feeder, I occasionally observed tropholaxis between a worker and an adult sexual on the feeder. This suggests that returning foragers possibly attend adult reproductives.

Whether or not time to unload influences recruitment rate in *P. ceres*, as it does with honeybee foragers (Seeley and Tovey 1994), is still unknown. If the workers are unloaded faster when reproductives are present in the colony this could help to increase

the turnaround time to the carbohydrate feeder. This faster unloading time, in turn, could increase recruitment rate as it does in honeybees (Seeley 1992; Seeley 1993).

On an ultimate level, the adult reproductives are not expendable but one could argue that larvae, at least in part, are. If a colony is food-stressed, it would benefit from the workers feeding the adults prior to larvae. If the larvae die, the colony fails to grow but the surviving work force can maintain the colony until food becomes more abundant. Indeed, in some cases larvae have been sacrificed in order to feed other colony members (Tschinkel 1993). In addition, the wealth of sex ratio literature (which is beyond the scope of this paper to review) suggests that life as a larva is not 100% guaranteed. Workers sometimes cannibalize larvae differentially according to sex (Passera and Aron 1993; Aron et al. 1994; Passera and Aron 1996; Sundstrom et al. 1996; Chapuisat et al. 1997; Aron et al. 2001). By contrast, the adult reproductives are the embodiment of the reproductive investment of a colony. The colony should go to great lengths to insure their investment survives until the mating flight. Since adult reproductive survival is a much higher priority than larval survival in a colony, it might be beneficial to the colony if adult reproductives influence workers' foraging decisions.

Eusocial colonies cannot afford to have larvae controlling worker foraging decisions. In wasps (Hunt et al. 1996; Hunt et al. 1998), bees (Winston 1987) and ants (Wheeler and Nijhout 1981; Wheeler 1986), larval nutrition has a direct effect on caste determination. In *Pheidole* the fate of an individual is decided at the larval stage (Wheeler and Nijhout 1981). This gives adult workers direct control over the production of minors, majors and reproductive females produced by the colony. If the larvae had control over the foraging decisions, they could determine their own fates regardless of the

benefit to of the colony (Reuter and Keller 2001). This could easily result in the breakdown of a eusocial colony's caste system. As long as workers are able to deprive larvae of certain nutrients, control of caste development can be maintained (Hunt et al. 1998).

P. ceres must be using an indicator other than larval needs to decide what nutrients are needed by the colony. One possibility is that ants are using the nutrients contained within themselves as a cue. Sorensen (1985), found that colonies of *Solenopsis invicta* containing hungry nurse ants are more likely to forage than colonies with satiated nurse ants. Hungry nurse ants could be depleting the reserves of the foragers and thus stimulating them to forage. In *Leptothorax albipennis,* lipid content in foragers has been shown to correlate with the likelihood of an individual to forage (Blanchard et al. 2000). The authors suggested that corpulence is a possible cue for workers to forage. In my study, there was additional supporting evidence that workers self-assess. In the brood switching experiment, workers in the subunits fed on carbohydrates foraged significantly less then those fed proteins. Workers might be using their internal stores of carbohydrates to determine whether or not to forage.

General conclusions

Workers of the ant *Pheidole ceres* appear to adjust their foraging preferences according to the colony's needs. This allows the colony as a whole to behave in a similar manner as a larger temperate animal, and to cycle between reproduction and survival strategies from year to year. During the spring the colony is in a reproductive phase. Food availability is high and the temperatures are favorable for *P. ceres* foragers. These

conditions might allow the colonies to forage for longer periods of time. In the fall there is a switch to a survival strategy. The preference for carbohydrates stays high despite the presence of larvae. The colony as a whole seems to be responding to long-term predictable changes but not adjusting to smaller less predictable changes.

The results of this study clearly show that workers are not responding directly to the nutritional needs of the larvae. Thus, the colony can maintain strict control over the developmental fate of each individual by depriving the larvae control over the foraging decisions. However, adult sexuals are able to increase the colony's intake of carbohydrates. The developmental fate of adult reproductives has already been determined and the cost of their influence to the colony as a whole is minimal. Instead, it would be advantageous to the colony to allow adult reproductives some control, to insure the survival of the colony's reproductive investment. When the adult sexuals are not present, the workers are apparently using a cue other than larval needs to determine whether or not to forage for a particular nutrient, possibly, what they are storing in themselves. Thus, larval nutritional needs are only met by an indirect mechanism such as the stores of tending nurse ants.

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Chapter 3 The nutritional ecology of *Pheidole ceres*: A measure of internal and external food stores in a seed-harvesting ant.

Abstract

In temperate climates, many social insect colonies must simultaneously reproduce, grow and collect enough stores to survive the winter. In order to balance the nutrients needed, individual workers should use a simple but reliable cue to determine the colony's nutritional needs. To examine the possibility that internal cues could be used, I measured the quantity of carbohydrates, lipids, amino acids and proteins stored in individual ants from ten colonies taken once a month from April to October. In addition, the seeds the *Pheidole ceres* colonies stored were analyzed for their nutrient content. Seeds serve as the primary lipid and protein storage vessels whereas the workers store amino acids and carbohydrates.

The relative nutrient levels were compared to the known foraging preferences of *P. ceres* at different times of the year. The levels of carbohydrates and amino acids matched the foraging preferences for carbohydrates and proteins found by Judd (in prep) respectively. This pattern suggests that the ants use these nutrients as indicators for colony nutritional state. I compared my results with studies that examined nutrient levels in non-seed harvesting ants and found evidence that life histories influence which nutrients would be the most reliable indicators of a colony's nutritional needs

Introduction

Ideally, animals living in temperate zones reproduce when the climate is favorable for foraging and focus on survival and food storage when the 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 the products of plant reproduction (nectar, pollen and seeds) are faced with a challenge because the animals' food sources are also taking advantage of the favorable climate for their own reproduction and growth. For these animals, seasons that are ripe for reproduction are usually the best times to acquire food necessary for storage. Individuals dependent on seeds, fruit and nectar not only have to maximize their reproduction during favorable times but budget in the time and energy necessary for food storage.

Many social insects appear to toggle between reproduction and storage. Annual colonies such as social wasps and bumblebees start new colonies each year (Wilson 1971; Michener 1974). Initially, colonies collect protein sources and high-energy foods (carbohydrates and lipids) to rear new workers and reproductives. Once the reproductive adults emerge, the colonies focus on collecting nectar presumably to allow the reproductive females to build up food reserves in order to survive the winter (West-Eberhard 1969; Hoshikawa 1981; Tsuchida 1991; O'Donnell 1998).

Seed harvesting ants and honeybees are simultaneously engaged in both food storage and reproduction. Honeybees collect excess amounts of pollen and nectar, much of which is stored in cells, to insure colony survival during the low productive periods (Waddington 1987; Seeley 1997). Ants do not construct specialized storage containers

but instead have other internal and external mechanisms to store food. All ants are able to store nutrients internally in their crops, fat stores and 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 the internal food stores and subsequently the foraging behavior of seed-harvesting ants has yet to be explored.

Since the colony as a whole is involved in several foraging strategies, there should be some mechanism for an individual forager to assess the needs of the colony. This mechanism would allow the colony to allocate its limited foraging force efficiently so that it only gathers the nutrients needed by the colony. Worker ants do not respond directly to the needs of the larvae (Cassill and Tschinkel 1995; Judd in prep). Instead, workers must rely on another cue to determine the needs of the colony. One possibility is the nutritional stores within the ants themselves (Blanchard et al. 2000). It has been shown that the 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). Since these levels vary then they are potential cues for workers to assess the nutritional status of the colony. Blanchard et al (2000) found that the levels of lipids in *Leptothorax albipennis* corresponded to the ants' foraging behavior. Foragers were shown to have lower levels of lipids than the non-foragers. In seed-harvesting ants, the nutrients stored by the colony are not only located in their internal stores but also in the seeds collected. Thus, the ants should only rely on the 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 *Pheidole ceres,* a small ant that lives in the Rocky Mountains. This ant forages for seeds and other food types. Judd (in prep) demonstrated that *P. ceres* adjusts its food preferences during different times of the year. Protein and carbohydrates are both collected equally during the spring. In the summer and fall carbohydrates are preferred to protein. 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 then compared these results to the foraging behavior observed by Judd (in prep) to see if ant nutrient levels correlate with ant foraging behavior. This study not only demonstrates what nutrients are being stored by *P. ceres* at different times of the year, but elucidates a possible cue foragers could use to determine the colony's needs.

Methods

Study organism

The colonies of *Pheidole ceres* used in this study are found in the Rocky Mountains (Larimer County, Colorado) at about 7,500 ft above sea level. The colony goes through three major stages during the year: 1) The production of sexuals and workers, 2) care of adult sexuals and 3) the rearing of worker larvae. For more details on the natural history of *P. ceres* see Judd (in prep).

Collections

Small samples of ants were collected with an aspirator from 10 wild colonies and frozen at –80°C. I collected 2 majors, 6-10 minors and a few adult sexuals, when present,

from each colony on the first of the month from April 2001 until October 2001. This amount of ants was chosen to minimize the impact on the colony.

Seeds were also randomly gathered as the ants were being aspirated. The seeds were separated by morphological traits and frozen at –80°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). The wet weight of each ant was determined. The dry weight was not determined for these ants because they were further tested for their nutrients.

Individual ants were homogenized in 150µl of distilled water and centrifuged at 14000/min. The four nutrients were measured (see below) in the following order: 1) protein and free amino acids, 2) carbohydrates and 3) lipids. All individuals from a single colony were analyzed simultaneously to control for between-trial variation.

Soluble Protein and Free Amino acids

I used the florescamine assay, described by Böhlen et al. (1973), to estimate total soluble proteins and total free amino acids with the following adjustments. 50µl of the aliquot was taken. Each sample was split into four equal portions. I added 12.5µl %5 Trichloroacetic acid (TCA) to two of the portions, which precipitated out the proteins, to determine total free amino acids. I added 12.5µl of distilled water to the other two portions. This provided a measure of total amine groups, which includes proteins and total free amino acids. Due to the sensitivity of this assay, I had to cut all of the previous

measurements in half when analyzing majors and males and fourfold when analyzing gynes. To all samples (regardless of the caste) I added 1.45ml of 0.1M Boric acid and then mixed in .5ml Fluoran (0.3mg fluorescamine/ ml acetone). Each sample was measured on a Fluorometer at 485nm (Excitation filter= 390nm and no emission filter). The samples were compared to a 40 nmol standard (982µl 5%TCA + 8µl 10mm 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) with the following adjustments. To 50µl of ant aliquot, I added 12.5µl 18% Na_2SO_4 and 1.25 ml of anthrone reagent. The sample was heated at 100°C for 12 minutes and then cooled to room temperature. The absorbance of the sample was measured at 625nm with a spectrophotometer and compared to the absorbance of 0, 5, 10, 15, 25, 50 ug glucose/ µl H_2O standards. This provided me with an estimate of micrograms of carbohydrates for each ant.

Lipids

Total lipids were measured with the phosphovanillan assay as described by Barnes and Blackstock (1973) and Wheeler and Buck (1992) with the following adjustments. 50µl of 1:1 Chloroform:methanol was added to the remaining ant sample. The mixture was vortexed and centrifuged for 2 minutes at 14000/min. The chloroform portion was separated from the rest of the sample and dried. The water-soluble half was added to the dried sample in case any lipids were left behind. To each sample, I added

200μl of con sulfuric acid and heated the sample at 100°C for 10 minutes. Once removed, 3ml of Vanillin reagent were added and the sample was allowed to develop for 30 minutes. The absorbance was measured at 525nm on a spectrophotometer and compared to the absorbance of 0, 18, 45, 72, and 90ug corn oil in1:1 chloroform: methanol standards (Barnes and Blackstock 1973; Wheeler and Buck 1992).

Seed Nutrient Analysis.

The nutrient content of the seeds commonly harvested by the ants was measured with the same procedures as the ants except for 2 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 seeds I collected varied in both size and number.

Data analysis

For each caste, I combined the three measurements for head width, pronotum width and wet mass in a principle component analysis. The resulting factors, used to represent body size, were compared to the nutritional measurements to look for possible correlations between physical measurements and nutritional measurements. I used parametric tests for worker castes and nonparametric tests for sexuals due to the small sample sizes in the sexuals.

Since the individual ants came from one of ten colonies and therefore not independent samples, I used the proc mixed procedure in SAS followed by a Tukey analysis to analyze the change in nutrients in ants across the months. Month was

considered a fixed effect, while colony of origin was considered a random effect. Each nutrient was analyzed separately for minors and majors. A total of 351 minors and 96 majors was analyzed. The change in nutrient levels in males and gynes was analyzed with the Mann Whitney Test due to the small sample sizes.

Results

General measurements:

The three physical measurements, head width, pronotum width and wet mass were combined in a principle components analysis. This analysis produced a single significant factor, which loaded positively for all three variables for minors, majors and males (Table 1). However, the first principle component did not include pronotum width in gynes. This created a variable that represented body size. For all castes, the first principle component showed no significant relationship with any of the four nutrient groups measured in this study. I also found that pronotum width was not correlated with any of the nutrients analyzed in gynes. Thus, there was no significant relationship with body size and the nutrient measurements.

Changes in nutrient levels during the year

Minors and majors

Both minors ($F= 2.91$, $P= 0.0188$) and majors ($F =5.99$, $p= 0.0002$) showed significant changes in carbohydrate levels from April through October. In the minors, the levels of carbohydrates started low in the early spring, rose during June and were

Table 1. Principle components analysis of the physical and nutritional attributes measured for *P. ceres* minors, majors, Reproductive females (gynes) and males. Asterices indicate the variable is explained by that factor.

	Minors	**Majors**	**Gynes**	**Males**
Attribute	**Factor 1**	**Factor 1**	**Factor 1**	**Factor 1**
Head Width	0.897852 *	0.885331*	-0.821588*	.901777*
Pronotum Width	0.820925 *	0.859624*	0.533162	.959059*
Mass	0.787661 *	0.801420*	-0.883907*	.926682*
Eigenvalue	2.448242	2.165045	1.705604	2.591735
Percent of total Variance	70.01%	72.17%	56.85%	86.39%

significantly higher in July. A significant drop in carbohydrate levels occurred between July and August and levels of carbohydrates in the ants remained low in September and October (Figure 1A).

Relative levels of carbohydrates were similar for the majors in the spring. However, during the transition from July to October the overall drop in the amount of carbohydrates in the majors was significant but it occurred much more gradually than in the minors (Figure 2A).

Lipids showed no significant changes in minors ($F= 1.19$, $P= 0.332$ Figure 1B) or majors ($F= 0.98$, $P= 0.453$ Figure 2B) throughout the year.

Overall, there was a significant change in the amount of free amino acids present in both minors ($F= 3.11$, $P= 0.0158$) and majors ($F= 2.55$, $P= 0.0315$) across the months analyzed. As with the carbohydrate levels, the levels of amino acids were low in the spring and showed a significant increase through June and July. However, the levels of amino acids remained high through August and then showed a gradual, significant drop through September and October (Figure 1C).

The levels of amino acids in the majors showed a similar pattern to the levels in the minors but the increase in amino acids levels into July was not significant. However, the drop in levels of amino acids from July through October was significant (Figure 2C).

Proteins showed very little change in either minors ($F= 1.30$, $P= 0.294$; Figure 1D) or majors ($F= 1.84$, $P= 0.122$; Figure 2D).

Figure 1: The change in A) Carbohydrates, B)Lipids, C) Free Amino Acids, D) Protein in *Pheidole ceres* minors collected during the colony's active season in 2000. The letters indicate the results of a Tukey analysis following an ANOVA (mixed procedure in SAS). Points with different letters indicate the two points are significantly different. N=354 for all four analyses.

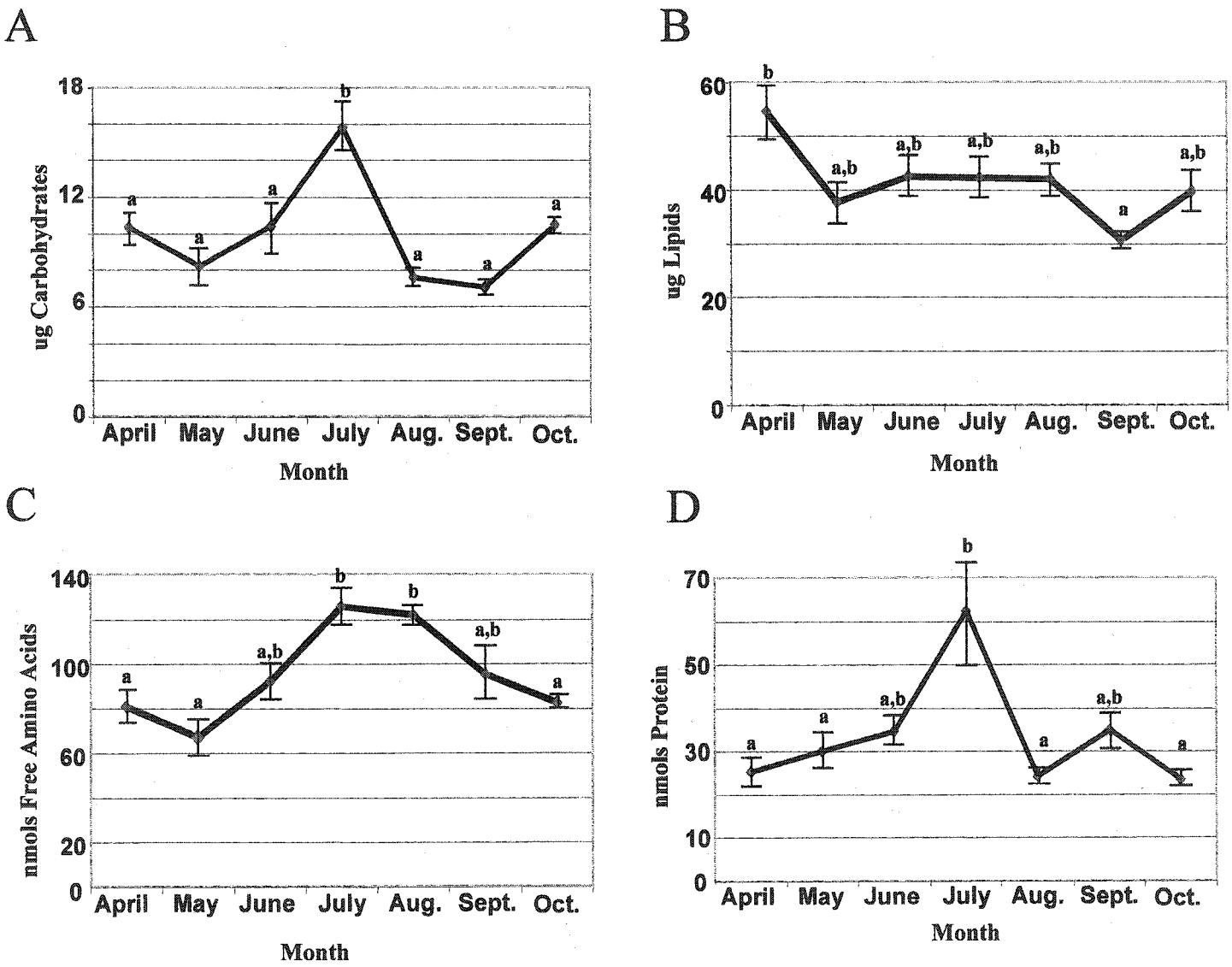
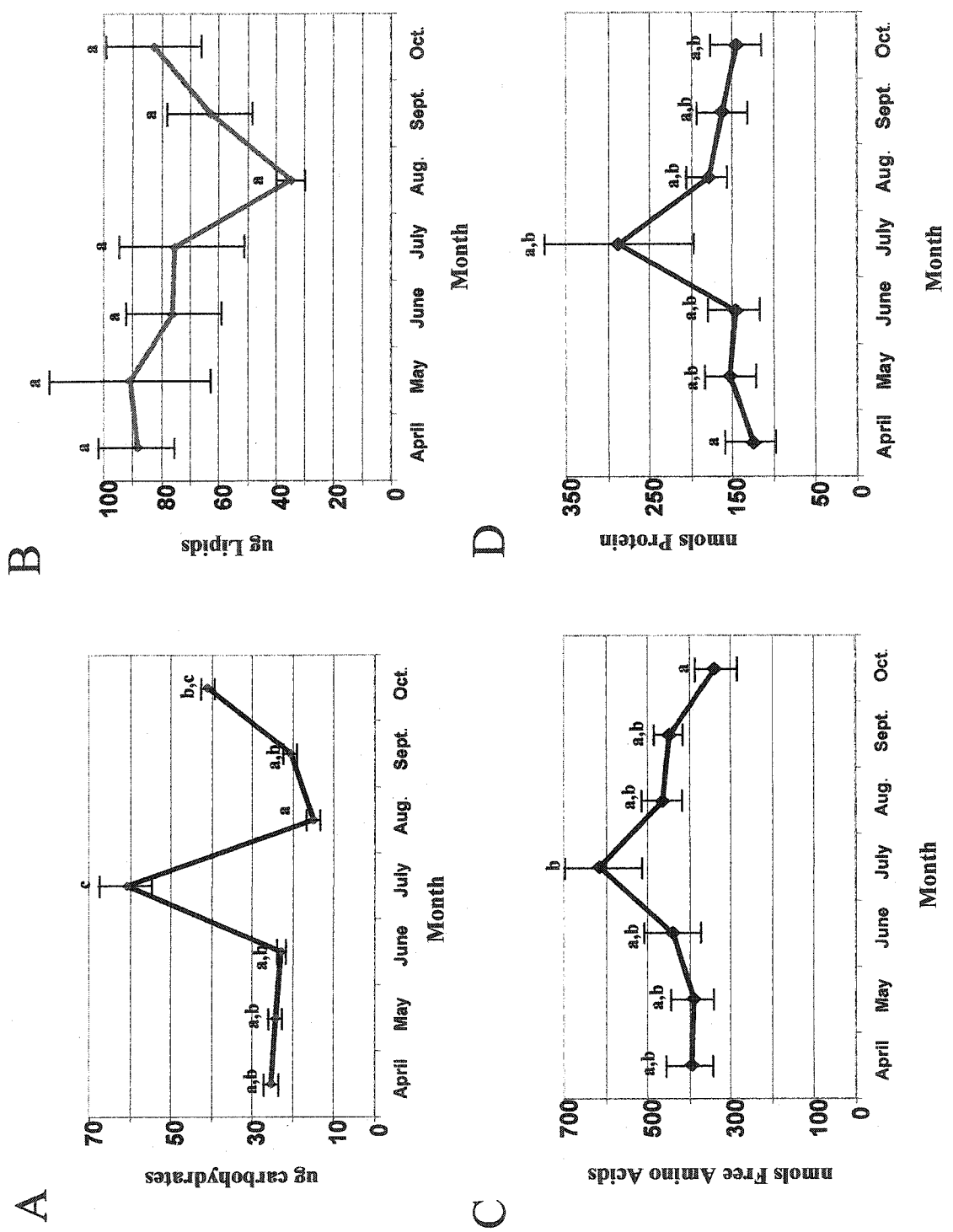


Figure 2: The change in A) Carbohydrates, B)Lipids, C) Free Amino Acids, D) Protein in *Pheidole ceres* majors collected during the colony's active season in 2000. The letters indicate the results of a Tukey analysis following an ANOVA (mixed procedure in SAS). Points with different letters indicate the two points are significantly different. N=96 for all four analyses.



Reproductive adults

The reproductive males showed a significant increase in the amount of carbohydrates from July to August ($T= 24$, $N_{july}= 6$, $N_{aug.} = 3$, $p< 0.05$; Figure 3A). However, the reproductive females showed a significant drop in carbohydrates levels from July to August ($T= 18$ $N_{july} = 4$ $N_{aug} = 2$, $p<0.001$; Figure 3A).

There was a significant increase in lipid content from July to August in reproductive females ($T= 11$, $N_{july} = 4$, $N_{aug} =2$, $p< 0.05$; Figure 3B). Unlike the female reproductives, the males did not show a significant change in lipid levels (Figure 3B). Both reproductive males and reproductive females showed no significant changes in the levels of amino acids (Figure 3C) or in the levels of protein (Figure 3D).

Seeds

Types of seeds collected

The five species of seeds found more than once in colonies of *P. ceres* were from: sunflower, *Helianthus annuus*; needle grass, *Stipa spp*; cinquefoil, *Potentilla glandulosa*; pigweed, *Chenopodium album*; and sagebrush, *Artemisia tridentata*. The quantity of seeds found in different months is shown in Table 2. Sagebrush, needle grass and pigweed were the predominant seeds collected by the ants (Figure 4). Cinquefoil seeds were only collected in abundance in May possibly because *Potentilla glandulosa*, a spring blooming plant, only produced seeds in May (Figure 4). There were 25 other seed species ("other seeds" in Figure 4) found in the colonies I examined. All of these were only found once. Based on my sample, sagebrush seeds were the most common seeds collected by *P. ceres*. Sagebrush is one of the predominant plants in this environment so it is not surprising that the *P. ceres* collected many seeds from these plants. The relative

Table 2 The numbers and average mass of seeds and pollen found in random samples taken from ten colonies of *Pheidole ceres* throughout the year. .

Seed Type	Number found	Avg mass (µg)
Sage Brush, *Artemisia spp.*	**248**	**6.2**
Grass, *Stipa spp.*	**78**	**10.0**
Lamb's Quarter, *Chenopodium album*	**48**	**35.0**
Sunflower, *Helianthus annuus*	**15**	**74.5**
Cinqfoil, *Potentilla grandulosa*	**23**	**7.0**
Bitterbush, *Purschia tridentate.*	**5**	**NA**
Other Seeds (1 seed found per species)	**39**	**NA**
Pollen Grains	**20**	**NA**

Figure 3: The means and SE of the amount of A) Carbohydrates, B)Lipids, C) Free Amino Acids, D) Protein found in *Pheidole ceres* adult males and female alates in July (just emerged) and August (Just before they flew). The bars with brackets indicate significant differences resulting from a Mann Whitney Test; * $p< 0.05$, ** $p< 0.01$. For females $N_{July}/ N_{Aug} = 4/2$; For males $N_{July}/ N_{Aug} = 6/3$.

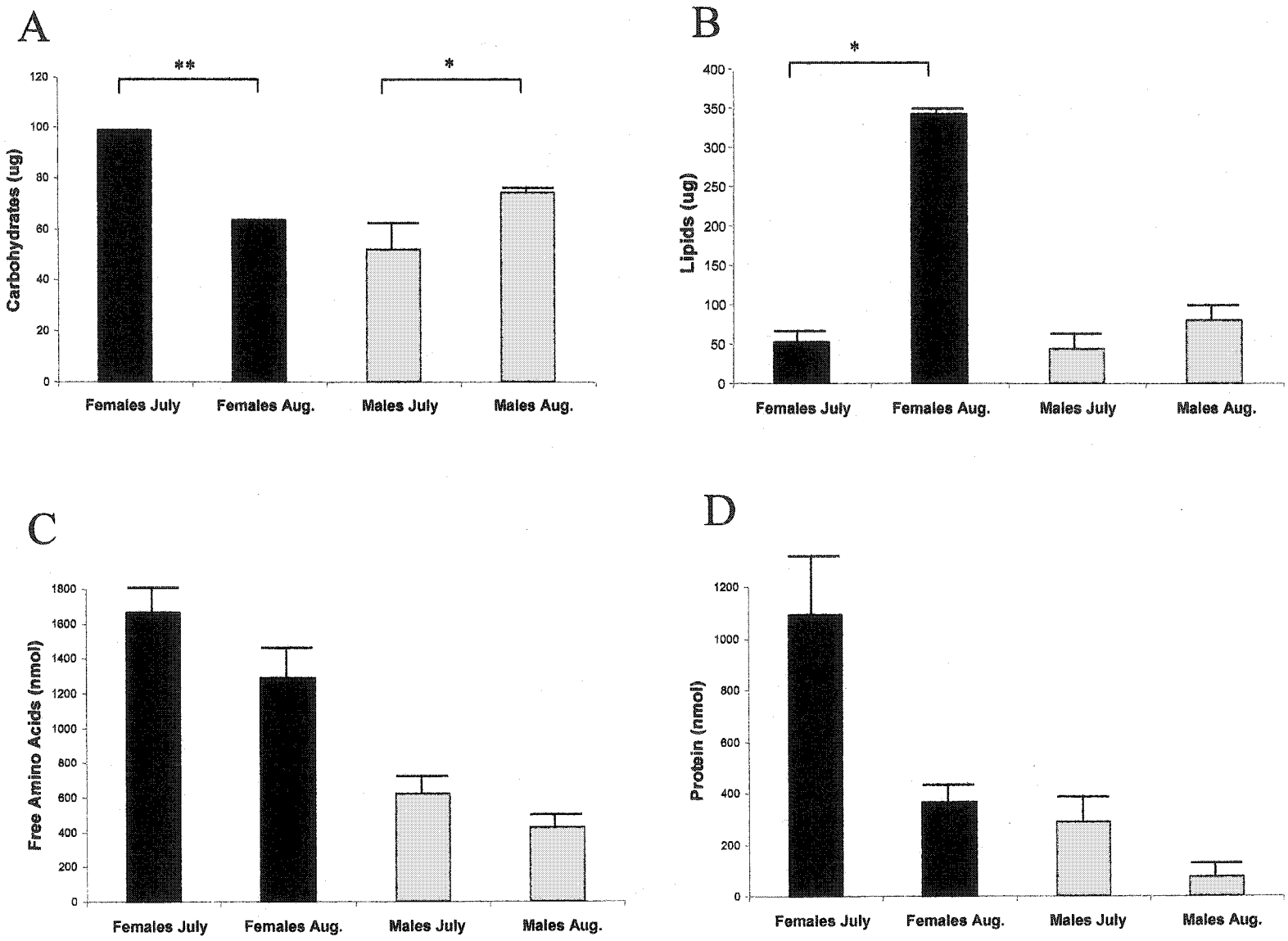
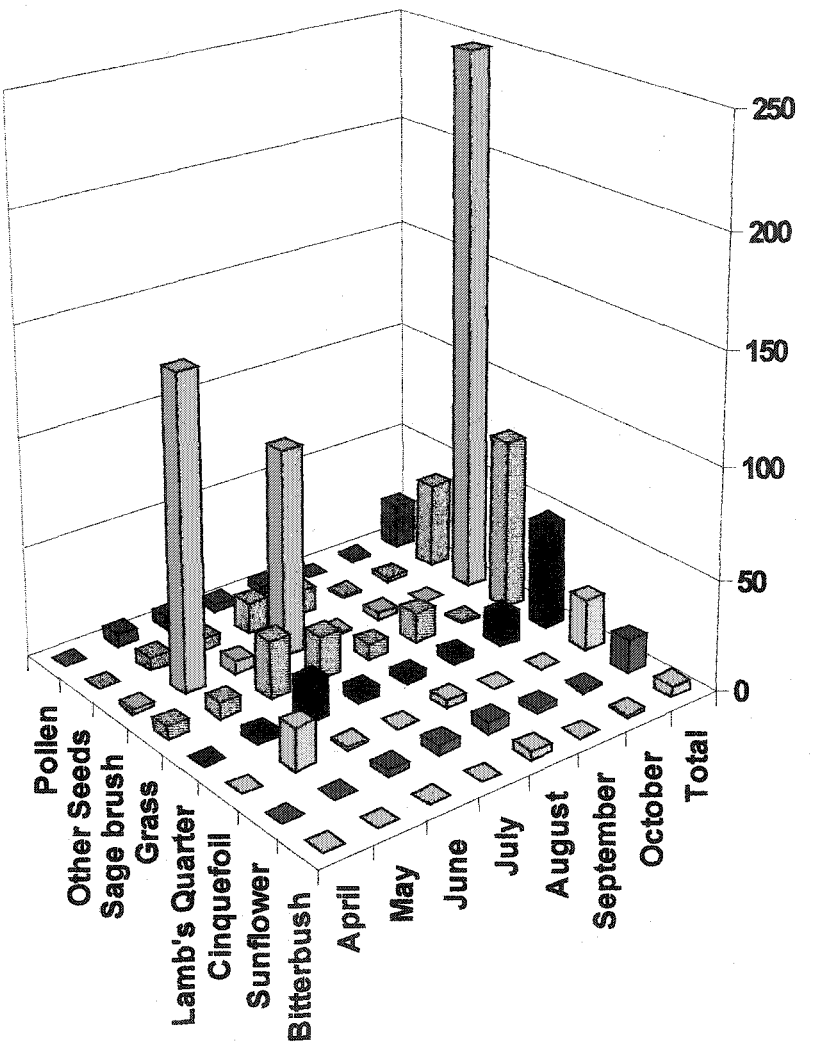


Figure 4: The number of different seed types collected at different times of the year from 10 *Pheidole ceres* colonies.



distribution of the other plants still needs to be determined to see if their relative abundance matches the proportion of seeds found in the colonies.

In addition to seeds, I found many pollen grains (Figure 4) in the samples I took. These were angiosperm pollen grains but the actual species of the pollen is unknown. I also found the remains of insects including ants, beetles and homopteran nymphs.

Seed nutrients

The nutrient levels found in the five major seeds are shown in Table 3. In all cases, the seeds have a large amount of lipids. Seeds of sagebrush, pigweed and sunflower also contain protein whereas needle grass and cinquefoil seeds appear to lack protein. Sagebrush seeds contain the highest amount of protein relative to their weight than every other seed. Based on this analysis, sagebrush seeds are the most nutritious seeds collected by *Pheidole ceres*.

Discussion:

The results of the nutrient analysis in *Pheidole ceres* show the following three patterns: 1) The adult reproductives showed significant changes in carbohydrates and lipids but not in proteins or amino acids; 2) Workers showed significant changes in carbohydrates and free amino acids but not lipids or proteins; and 3) All of the seeds commonly collected by the ants all were high in lipids but only two, sagebrush and pigweed, contained a high amount of protein.

Table 3: The means (SE) of the nutrients found in seeds collected from colonies of *Pheidole ceres*. N are for carbohydrates & Lipids / Amino acids and Proteins.

Seed Type	N	Carbohydrates ug	Lipids ug	Amino Acids nmol	Estimate of Amino Acid mass in ug*	Protein nmol
Sage Brush	40/8	0.0487 (0.0245)	14.196 (2.095)	13.425 (4.075)	1.874	10.825 (3.925)
Grass	8/4	0.240 (0.158)	16.934 (3.140)	39.175 (6.186)	5.469	0.000 (0.000)
Lamb's Quarter	4/4	1.559 (0.436)	49.207 (2.653)	30.200 (4.400)	4.216	23.900 (10.900)
Sunflower	2/1	2.583 (1.722)	227.272 (177.332)	19.600 (0.000)	2.2736	13.500 (0.000)
Cinqfoil	1/1	0.000 (0)	12.240 (0.0)	18.400 (0.000)	2.569	0.000 (0.000)

* Estimate based on the average mass of all 20 amino acids = 0.1396ug/nmol

Nutrient changes in adult reproductives

The changes in carbohydrate and lipid levels were very different for male and female reproductives. Carbohydrate levels increased in males from July to August but decreased in females during that same period. Unlike males, female lipid levels increased during July, suggesting that the females converted their carbohydrates to lipids for long-term storage. Males just need enough energy reserves to locate a mate and do not need long-term lipid stores, explaining the lack of any increase in lipid content in males. A similar pattern of lipid change in reproductive adults was found in *Tetramorium caespitum*. Males showed a slight decrease in lipids while the females showed a significant increase in lipid levels (Peakin 1972).

The adult reproductives showed no significant change in proteins or amino acids. Although adults do not require protein for growth, this result is still surprising because female reproductives use storage proteins (Wheeler and Martinez 1995) and need protein for egg production. The failure to detect an increase in protein levels in reproductive females suggests that storage proteins are acquired as larvae. Vinson (1968) demonstrated that males and female alates of *Solenopsis invicta* gained all of their protein stores prior to emergence. If the adult reproductives do build up protein stores as larvae, the demand for protein by the colony as a whole would increase. Thus, the preference of a colony for protein should increase when adult reproductives are being reared. This hypothesis is consistent with the foraging behavior observed by Judd (in prep), which demonstrated that the highest preference for protein in *P. ceres* colonies was during May when reproductive-destined larvae were present.

Food storage in Pheidole ceres

Food stores in *Pheidole ceres* seem to exist in three forms: long-term storage in seeds, short-term reserves in majors and immediate reserves in minors. Seeds are a perfect long-term food storage vessel. The seed itself is an adaptation for storing food for the plant embryo and ants are just commandeering this feature for their own purposes. Seeds provide a source of protein and lipids for the winter. *P. ceres* specializes on seeds from only a few species. The seeds fall into two categories, seeds high in lipids and seeds with protein and lipids. Sagebrush, *Artemisia tridentata,* the most commonly collected seed type, has the highest percentage of protein than all of the other seeds collected and represents 52% of the total seeds sampled. Whether or not *A. tridentata* is actually preferred to other species by *P. ceres* remains to be seen. Sagebrush is a very common plant in *P. ceres'* habitat and it may be that *P. ceres* just encounters that seed type more often than others.

The minors showed the greatest fluctuations in amino acid levels and carbohydrate levels relative to the other castes but not in lipid or protein levels. As with other species of *Pheidole* (Wilson 1984), the minors are probably the primary caregivers in *P. ceres*. Castes involved in colony care are continuously exchanging food with other colony members (Vinson 1968; Brian 1977; Cassill and Tschinkel 1995; Cassill et al. 1998; Cassill and Tschinkel 1999). One explanation for the greater fluctuations in carbohydrates and amino acids is these nutrients are allocated to other colony members first. They are easier to utilize and are less efficient for storage when compared to proteins and lipids. The changes in minors' nutrient levels represent the changes in

immediate needs of the colony. The fact that lipid and protein content showed little change in the minors might be explained by the fact that seeds and majors are two backup sources for these nutrients.

The majors' nutrient content fluctuated, but not as quickly as the content of the minors, suggesting that the majors' food stores are being depleted less rapidly than the minors' food stores. There are many species of ants in which majors act as food repletes (Wilson 1974; Lachaud et al. 1992; Tschinkel 1993). In *Solenopsis 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. However, the fact that majors are larger than minors means there is a larger amount of nutrients per major. *Pheidole ceres'* seed storing behavior might explain the discrepancy between lipid content of majors of other ants and majors of *P. ceres*. Non-seed storing ants appear 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 buffers non-harvesting ants during a food shortage. If the colony harvests seeds, then the role of a major replete becomes redundant. If this hypothesis is true, majors of other seed harvesters should also have reduced their role as food repletes (but see Lachaud et al. 1992).

It would be interesting to examine the levels of nutrients of fungus farming ants such as *Atta*. Since they have an ample food supply at all times, repletes should be non-existent. The lack of repletes could help explain why leaf-cutting ants have never colonized the colder temperate environments. They have lost their ability to create long-

term food stores. If this is true, than the food stores in fungus harvesting ants should be constant for all nutrients.

Relation of internal food stores to foraging behavior

Judd (in prep) found that the feeding preference of *P. ceres* varies throughout the year. In the spring, the ants showed no preference for protein or carbohydrates. In the late summer and in the fall the ants preferred carbohydrate sources to protein sources. When I compared the nutrient levels of the ants with this foraging pattern an interesting pattern emerged. The levels of the carbohydrates and amino acids found in *P. ceres* workers correlated with the carbohydrate and protein preferences shown by the ants and thus could have provided workers with reliable cues for their colony's nutritional needs. In the spring, both the amino acid levels and carbohydrate levels were low, meaning the colony was hungry for everything. At this time, *P ceres* foraged for all nutrients equally. In the fall, the levels of carbohydrates were as low as they were in the spring, but the levels of amino acids in the colony were high. Under these conditions, the colonies preferred carbohydrate sources to protein sources. During these two time-periods, the level of amino acids was negatively correlated with the protein preference, suggesting that the ants could rely on amino acid levels to determine the colony's protein needs.

At first glance, the correlation between carbohydrate levels and carbohydrate preference appears to have broken down in early July because both carbohydrates and amino acids levels were high and the ants showed a high carbohydrate preference (Judd in prep). However, Judd (in prep b) has demonstrated that adult reproductives increase the carbohydrate preference in workers. In this study, I have demonstrated that both

males and female reproductives increased their energy reserves during their stay in the colony. Thus, the reproductives acted as a carbohydrate sink, driving the workers to seek out more carbohydrates. The sharp decrease in carbohydrate levels in the minors from July to August supports the idea that that demand for carbohydrates was extremely high during this time. It appears that the feeding of adult sexuals can drain a colony's carbohydrate stores. Interestingly, there were lower numbers of larvae in the colony during this time. The lack of hungry larvae was consistent with the maintenance of high amino acid levels in the colony from July to August. There was little demand for amino acids or proteins at that time. During July, *P. ceres* colonies appeared to focus on reproduction, while growth seems to be suspended for a month. The colony appears to have a hierarchical system, placing reproduction at a higher importance than growth.

Blanchard et al. (2000) found evidence that in the ant, *Leptothorax albipennis*, nutrient levels may stimulate foraging. In their study, foragers had lower lipid levels than non-foragers. They suggest that low lipid levels stimulate individuals to forage. These results suggest that if ants use nutrient levels to make foraging decisions, different species might cue in on different nutrients.

The key difference between the life histories of *P. ceres* and *L. albipennis* is that *P ceres* harvests seeds while *L. albipennis* does not. Protein and lipids showed very little variation in *P. ceres* and are readily available in the seeds they collect. Seed harvesting has two consequences, 1) workers of *P. ceres* may forgo storing lipids and proteins in favor of the smaller nutrients; and 2) The amount of protein and lipids stored by the colony are difficult to determine by an individual because ants can not estimate seed number. These consequences would favor the smaller nutrients, carbohydrates and amino

acids as foraging cues. *L. albipennis* depends solely on their internal food stores for lipids and proteins so these two nutrients would be reliable indicators of a colony's needs. Carbohydrates are probably used immediately by the workers or quickly turned to lipid reserves. If the differences in life histories between these ants are responsible for these differences than other, non-seed harvesters should show greater fluctuations in lipids and protein content than in amino acid and carbohydrate content.

The above prediction is supported by Ricks and Vinson (1972). They examined the protein, lipid and carbohydrate contents of *Solenopsis invicta* colonies over an entire year. Indeed, the protein and lipid levels showed more fluctuation than the carbohydrate levels in these colonies. The authors noted that the ants gathered many insects when the protein content was low, supporting the idea that non-harvesting ants might cue onto their internal protein and lipid stores.

General conclusions

The results of this study demonstrate that life history of the ants affect nutrient storage and possibly the evolution of foraging cues. Ants that harvest seeds use these as the main lipid and protein stores, reducing the need for individuals to act as repletes. Indeed, the majors of *P. ceres* did not show higher percentages of lipids or proteins. In addition, seed harvesting would allow the workers to store other important nutrients. The ability to store smaller nutrients could help to reduce the time it takes to raise larvae because smaller molecules would be processed faster. This increased access to smaller nutrients could speed up growth and reproduction. The increase in variety of stored

nutrients would be a valuable trait for ants living in temperate zones because the time available for growth and reproduction is short.

Seed harvesters may have evolved to cue in on carbohydrates and amino acids because these nutrients show greater and more reliable fluctuations than protein and lipids. Workers would be unable to assess the levels of lipids and proteins in the colony because these nutrients are stored in the seeds. Non-seed harvesters could use lipids and protein levels to determine the hunger state of their colony because workers only have to look to their internal stores. In this case, lipid and protein levels would be reliable indicators of colony hunger because they accurately reflect the colony's food stores. Low lipid and protein levels in these workers would indicate that the colony's cupboard is running dry and could stimulate foraging in workers deficient in these nutrients. Thus far, we only have correlations between nutrient levels and behavior. Studies that artificially manipulate levels of nutrients in ants will allow us to directly test these ideas.

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