Colony-level macronutrient regulation in ants: mechanisms, hoarding and associated costs

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Social and nonsocial organisms both require a suite of nutrients in correct amounts and ratios to promote growth and fitness, but the nutrient profiles of available foods are rarely optimal. Nutrient acquisition in insect societies is more complex compared to that of nonsocial organisms however, because foraging is restricted to only a proportion of the colony, and these members must satisfy their own nutritional requirements and those of other members having distinct nutritional needs. In this study we used laboratory colonies of the fire ant *Solenopsis invicta* to quantify how ants regulate their protein–carbohydrate intake when restricted to diets with different fixed protein–carbohydrate (p:c) ratios, and to quantify, at both the individual and colony level, behaviours and costs associated with nutrient regulation when feeding on these foods. We found that ants were most attracted to foods with equal or moderately protein-biased p:c ratios. However, colonies on these two treatments created large hoards, and the p:c ratios of these foods differed from that of collected food. In general, carbohydrates were extracted and protein was retained. As a result, carbohydrate intake on all diets except the extremely protein-biased diet was similar. However, carbohydrate regulation on diets with equal and moderately protein-biased p:c ratios may be costly through elevated worker activity and mortality, and through reduction of worker lipid reserves. For colonies feeding on heavily protein-biased food, energy production may have been achieved via gluconeogenesis. We discuss our results in relation to how dietary p:c imbalances in naturally encountered foods may be driving ant foraging behaviour in the field.
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Insect societies, especially ant colonies, have been described as superorganisms sharing many functional parallels with individual organisms (Wheeler 1911; Hölldobler & Wilson 2009). Among these is the fundamental need to acquire a suite of nutrients in the correct amounts and ratios promoting growth and fitness. However, there are a number of factors that make nutrient acquisition at the colony level more challenging compared to that for a solitary individual. First, division of labour in ant societies results in foraging responsibilities, and thus nutrient retrieval, being assigned to only a subset of adult colony members. Second, most ant colonies contain overlapping generations composed of mature and immature individuals having different nutrient requirements (Hölldobler & Wilson 1990; Cassill & Tschinkel 1999a). Therefore the challenge for foraging workers is that they must harvest food such that it satisfies their own nutritional requirements while also addressing the nutritional needs of the other members of the colony, including the queen, the larvae, and the other workers. An

additional consideration is foraging costs, including energetic expenditure and potential mortality of workers when foraging (Wolf & Schmid-Hempel 1990; Beauchamp 1992).

The decision of a forager to retrieve an encountered food item is shaped by both internal and external factors, occurring at both the individual and colony level. At the individual level, a worker's current physiological condition (e.g. endogenous stores of lipids; Blanchard et al. 2000) may prompt foraging behaviour, and attractiveness of an encountered food item may be affected by the perceived relative availability and/or abundance of food items (Stein et al. 1990; Hahn & Wheeler 2002; Kay 2004), and their novelty (Howard et al. 1996). At the colony level, feedback related to larval nutritional demands (especially for protein), transmitted through a 'chain-of-demand' between brood, nurse workers and foragers, and created through colony member food sharing is likely to be important (Cassill et al. 1998; Cassill & Tschinkel 1999b; Behmer 2009). However, the strength of both internal and external cues directing worker foraging decisions can vary temporally, compounding the task of nutrient retrieval especially in the face of potential resource shortfalls (e.g. seasonal variation in resource availability). Extensive research of ant nutritional biology has helped elucidate potential determinants guiding collection of

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resources by workers, including, but not limited to, resource preference of different colony members (Cassill & Tschinkel 1999b; Portha et al. 2004), temporal shifts in resource availability and worker preference (Cannon & Fell 2002), and food distribution among colony members (Vinson 1968).

Two critical nutrients that influence ant foraging behaviour and performance are protein and carbohydrates (Cassill et al. 1998; Cassill & Tschinkel 1999b). However, researchers often investigate the effects of these two nutrients in isolation from one another, even though all naturally available food items encountered by ants (including honeydew) contain a mixture of macro- and micronutrients. The key is that various foods contain different absolute amounts and ratios of nutrients, at both a spatial and temporal scale. Additionally, few studies of ant nutritional biology have linked changes in macronutrient availability to alterations of behaviour and physiological condition of individuals (but see Kay et al. 2006), and even fewer have focused on colony-level behaviours of ants associated with nutrient regulation in a framework of imbalanced nutrient availability, and the costs of these behaviour (but see Dussutour & Simpson 2008a, 2009).

How animals simultaneously regulate the intake of multiple nutrients, and how the nutritional content of food affects animal behaviour and physiology can readily be explored in an integrated fashion using the experimental approach of the 'geometric framework' (reviewed by Behmer 2009). A key strength of this approach is that it allows visualization of how an organism prioritizes the intake of particular nutrients when it is restricted to a food with a defined, and fixed, nutrient profile. In particular, this approach reveals the extent to which an organism will overeat nutrients present in excess of requirements, in order to increase their intake of nutrients that are in short supply. And when these compromises are measured across a range of foods with different nutritional profiles, insights about global rules governing nutrient regulation can be elucidated (Raubenheimer & Simpson 1999; Simpson et al. 2004). The geometric framework was originally designed for use with insect herbivores (reviewed by Behmer 2009), but it has also been used to study nutrient regulation in a broad range of organisms, including chickens (Raubenheimer & Simpson 1997), rats (Simpson & Raubenheimer 1997), mice (Sørensen et al. 2008), fish (Ruohonen et al. 2007), and even humans (Simpson et al. 2003). Quite recently it has also been used to study nutritional regulation in ants (Dussutour & Simpson 2008a, 2009).

In the current study we used the experimental approach of the geometric framework to explore nutrient regulation at the colony level in the red imported fire ant, Solenopsis invicta. We did this by giving experimental ant colonies ad libitum access to foods with different protein–carbohydrate ratios, then we quantified the amounts of food collected over a 7-week period and expressed these data in terms of protein–carbohydrate intake. We also investigated whether the amounts of protein and carbohydrate collected were equal to the amounts of these macronutrients consumed by the colony (see Dussutour & Simpson 2009). Furthermore, we quantified potential costs associated with procurement of food, including worker foraging activity, worker mortality and alterations to worker physiological condition (measured in terms of amounts of endogenous lipid stores). We discuss how results from our study may elucidate important mechanistic linkages associated with nutrient availability under more natural conditions.

METHODS

Experimental Colonies

We collected polygynous colonies of Solenopsis invicta from 10 source colonies at the Riverside campus of Texas A&M University,

U.S.A., between 19 April and 5 May 2008, and maintained them in their original nest soil in buckets for 2 days; during this time we provided colonies with fresh-frozen crickets, 20% (v/v) honey solution, and water. We then used a drip-floatation method (Banks et al. 1981) to remove workers, brood and queens from the soil, and then left these colonies separately in large tubs overnight before forming monogynous experimental colonies. At this time we also haphazardly collected small groups of workers from each source colony and placed them into vials containing silica gel desiccant in preparation for lipid extraction (see below).

Each monogynous experimental colony consisted of a single wingless queen, 1750 mg workers (haphazardly chosen), 250 mg larvae and 125 mg pupae. This generated a worker-to-total brood ratio of ∼5:1, which mimicked natural S. invicta colonies during mid-spring (Cassill 2002). Larvae and pupae included in experimental colonies ranged in size and developmental stage, but we purposely avoided selecting larvae and pupae of reproductives. The 10 sources colonies produced different total numbers of experimental colonies; often source colonies did not provide adequate amounts of workers and/or larvae (and in some instances numbers of queens) to generate five experimental colonies (one for each treatment). Nevertheless, each experimental treatment had experimental colonies from at least four source colonies.

Each experimental colony was housed in a 24.6 × 19.2 × 9.5 cm plastic box, provided with a 15 cm diameter lidded and covered petri dish for use as an artificial nest chamber, filled approximately half-full with hardened Castone® dental stone (Castone Corp., Opelika, AL, U.S.A.), and an ad libitum water source. Colonies were housed in an insectary at 26 °C, kept at ambient humidity of 45–60%, and maintained under a 12:12 h light:dark cycle (using fluorescent lighting). We kept the nest chamber humidity levels high by regularly moistening the Castone® substrate (Cassill & Tschinkel 2000).

Experimental Diets

Experimental diets consisted of five dry, granular synthetic foods modified from both Straka & Feldhaar (2007) and Dussutour & Simpson (2008b), and their total combined protein (p) and digestible carbohydrate (c) content ranged from 80 to 83%. The five diets, expressed as the percentage of diet total dry mass, were: (1) p14:c69, (2) p21:c62, (3) p41:c41, (4) p60:c20 and (5) p67:c13. The dietary protein component was an approximate 1:1 mixture of whey protein concentrate and calcium caseinate, with a small but constant amount of protein provided by whole egg powder (which was also a source of essential lipids, including sterols). The sole source of digestible carbohydrate (henceforth only carbohydrate) in our experimental diets was sucrose. The amount of each dietary ingredient used in making each experimental diet is shown in Table 1. According to product nutritional information, each component in Table 1 contained impurities that reduced the amounts of whey protein by 15%, calcium caseinate by 8%, and sucrose by 7%. We also added a small amount of methyl 4-hydroxybenzoate to each diet (0.5 mg) to retard microbial growth (Dussutour & Simpson 2008b).

We combined all the dry dietary components and homogenized them using an electric mixer. We dissolved water-soluble vitamins in water, and we mixed insoluble beta-carotene by shaking it vigorously for 30 s before adding it to dry components, and then added it to the original dry ingredients. After complete mixing, diets were spread evenly on a large plastic weighing dish and placed in an oven set at 35 °C until dry. We formed 1 mm diameter granules by grinding dried food through a no. 18 U.S.A. Standard Sieve. Workers of all body sizes readily collected this granule size in preliminary trials (personal observation).