Summer and fall ants have different physiological responses to food macronutrient content

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ABSTRACT

Seasonally, long-lived animals exhibit changes in behavior and physiology in response to shifts in environmental conditions, including food abundance and nutritional quality. Ants are long-lived arthropods that, at the colony level, experience such seasonal shifts in their food resources. Previously we reported summer- and fall-collected ants practiced distinct food collection behavior and nutrient intake regulation strategies in response to variable food protein and carbohydrate content, despite being reared in the lab under identical environmental conditions and dietary regimes. Seasonally distinct responses were observed for both no-choice and choice dietary experiments. Using data from these same experiments, our objective here is to examine colony and individual-level physiological traits, colony mortality and growth, food processing, and worker lipid mass, and how these traits change in response to variable food protein–carbohydrate content. For both experiments we found that seasonality per se exerted strong effects on colony and individual level traits. Colonies collected in the summer maintained total worker mass despite high mortality. In contrast, colonies collected in the fall lived longer, and accumulated lipids, including when reared on protein-biased diets. Food macronutrient content had mainly transient effects on physiological responses. Extremes in food carbohydrate content however, elicited a compensatory response in summer worker ants, which processed more protein-biased foods and contained elevated lipid levels. Our study, combined with our previously published work, strongly suggests that underlying physiological phenotypes driving behaviors of summer and fall ants are likely fixed seasonally, and change circannually.

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1. Introduction

Macronutrients (protein, carbohydrates, and lipids) are the key drivers of growth and reproduction for most animals (Simpson and Raubenheimer, 2012), but often the individual foods available to foraging animals contain macronutrients in suboptimal amounts and/or ratios (Behmer and Joern, 2012). More studies are showing that many animal species regulate their macronutrient intake (Behmer, 2009; Simpson and Raubenheimer, 2012), and that a given species regulates its macronutrient intake depending on its immediate physiological demands (Clark et al., 2013; Maklakov et al., 2008). Shifts in seasonality (e.g., photoperiod, temperature, water availability) are accompanied by some degree of change in the quality and abundance of foods available to animals. For example, in temperate grasslands, in the spring and summer, herbivores

encounter actively growing plants with high protein (p) and carbohydrate (c) content (Behmer and Joern, 2012; Lenhart et al., 2015). In contrast, as winter approaches, plants stop growing, and protein and carbohydrate content drops dramatically.

In response, animals are observed to respond, both behaviorally and physiologically, to these changes (Basson and Clusella-Trullas, 2015; Cook et al., 2011), which are often entrained to seasonal shifts in photoperiod, temperature, and associated environmental conditions (DeCoursey, 2004; Helm et al., 2013; Kumar et al., 2010), and that interact with circadian processes that operate on a broader timescale (i.e., circannually) (Danks, 2005; Saunders, 1977). For example, pregnancy or lactation in vertebrates is timed to periods when protein-rich foods are abundant (Bell et al., 2000; Dewey, 1997), while animals preparing to migrate or hibernate often encounter an abundance of high-energy food prior to periods of food dearth (Hernandez, 2009). However, prolonging nutrient availability past timing of these periods can delay expected behavioral and physiological responses (Humphries et al., 2003; Vuarin et al., 2015).

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For most animals the causal relationship between seasonal environmental cues, food abundance/quality, and changes in animal behavior and physiology remain unclear, in part because experiments that disentangle their individual effects are often difficult to conduct. Social insect colonies offer a number of characteristics that make them a tractable model for addressing this issue. First, a social insect colony has traits that can be considered analogous to a solitary animal. For instance, sterile workers and fertile queens are often compared to animal somatic and reproductive tissues, respectively (Seeley, 1995). Individual workers can be analogous to nutrient storage cells, holding lipid reserves or liquid carbohydrates (Hölldobler and Willson, 1990). These, and many other examples (Gillooly et al., 2010; Hölldobler and Wilson, 2009; Seeley, 1995; Shik et al., 2012) form the basis for many referring to a social insect colony as a 'superorganism' (Wheeler, 1928). Second, because an individual colony is often long-lived (Jemiality et al., 2005; Keller and Genoud, 1997), it will experience seasonal changes in photoperiod, temperature, and associated environmental conditions (e.g., changes in rainfall patterns), including associated shifts in the nutritional quality/abundance of food. Third, like solitary animals, social insect colonies practice selective feeding (Kay, 2004; Roces, 1990; Wilder and Eubanks, 2010), and tightly regulate their protein–carbohydrate intake (Cook and Behmer, 2010; Cook et al., 2010, 2012; Dussutour and Simpson, 2008, 2009). Ants utilize a broad range of different food types (Hölldobler and Willson, 1990), and fire ants exhibit seasonal changes in their behavior and physiology (Tschinkel, 1993) that are likely accompanied by shifts in food selection tied to regulating colony nutrient intake (Cook et al., 2011).

Previously we demonstrated fire ants collected in the summer and fall had distinct behavioral responses to food macronutrient variability that were independent of environmental cues (Cook et al., 2011). In that study polygynous field colonies were collected during summer and fall and brought to the lab, where they were then split into replicate experimental queen-right lab colonies standardized for size and demography. These experimental colonies were maintained under similar temperature, humidity, and light regimes, and both subjected to the same cafeteria-type experiments. The first experiment was a no-choice experiment, with colonies constrained to a single food with a fixed p:c ratio to observe how workers respond behaviorally to regulating colony nutrient intake when feeding on foods having protein–carbohydrate ratios that differ relative to self-selected p:c ratios. When the food p:c ratio was balanced (1:1), summer and fall colonies collected equal amounts of food. However, as the p:c ratio of the experimental foods became increasingly imbalanced summer colonies collected significantly more food. In contrast, fall colonies always collected the same amount of food, regardless of its p:c ratio. The second experiment was a choice experiment, which gauged self-selected p:c intake by providing colonies with nutritionally sub-optimal, but complimentary food-pairings. Both summer and fall colonies actively regulated their protein–carbohydrate intake and both selected carbohydrate-biased intake targets (~1:1.5). A significant observation made for both experiments was that summer ants consumed more carbohydrate when it was abundant relative to the protein need of the colony (Cook et al., 2011).

Following these findings, we predict summer and fall fire ants have distinct physiologies independent of environmental cues, underling their contrasting behavioral responses to variable food macronutrient content. Contrasting seasonal physiologies, either apart from or combined with nutrient deficits or excesses experienced by summer or fall colonies in our previous experiments (above), are predicted to have consequences for colony performance. The directionality of consequences are predicted to align based on the nutritional needs of summer and fall fire ant colonies outlined previously (Cook et al., 2011). Here, using data from those same experiments, we test these predictions by comparing changes in workforce and brood biomasses, worker mortality and lipid content, and how summer and fall colonies manipulated unconsumed foods having variable macronutrient content.

2. Materials and methods

2.1. Experimental fire ant colonies

For a more thorough description of methods used to establish lab fire ant colonies for the experiments detailed below, see Cook et al. (2011). Briefly, six polygynous summer and fall colonies were collected between June 5 and June 15, 2009, and between October 20 and October 31, 2009, respectively. Colonies were returned to the lab, and a drip-floatation method (Banks et al., 1981) was employed to remove workers, brood, and queens from nest soil. Experimental colonies consisted of a single wingless queen, 1000 mg (~500) workers (haphazardly chosen), 200 mg larvae, and 100 mg pupae. Larvae and pupae included in experimental colonies ranged in size and developmental stage, but larvae and pupae of reproductives were purposely avoided. To control for field colony effects, for each of the six colonies collected, one experimental colony (above) was randomly allocated to each of the eight dietary treatments (see below). Simultaneously, a subset of workers was haphazardly collected from each source colony for later lipid extraction (see below).

Individual experimental colonies were housed in a 24.6 cm × 19.2 cm × 9.5 cm plastic box, provided with a 15 cm diameter lidded and covered Petri dish filled approximately half-full with hardened Castone® dental stone as an artificial nest; the Castone® substrate was moistened regularly to maintain high humidity inside nest chambers. Water was provided *ad libitum*. Colonies were housed in an insectary under a 12 h:12 h L:D diel cycle (using fluorescent lighting), and kept at 26 °C and with ambient humidity (45–60%).

2.2. Experimental foods

Experimental foods consisted of five agar-based synthetic foods, which contained the same quantities of micronutrients, salts, vitamins, and lipids from whole egg powder, but varied in protein and carbohydrate content (Cook et al., 2011). The foods' protein component consisted mainly of a 1:1 mixture of whey protein and casein, and the carbohydrate component was sucrose. The following combinations of protein and carbohydrate, expressed as percentage of total food dry weight, represent the p:c ratios of the five experimental foods: (1) 54% protein and 18% digestible carbohydrate (p54:c18), (2) p42:c32, (3) p37:c37, (4) p33:c43, and (5) p19:c57 (see Table S1).

2.3. Experimental protocol

2.3.1. Food collection and consumption

No-choice and choice experiments were performed simultaneously over a five-week period. In the no-choice experiment, experimental ant colonies were restricted to one of the five diets. In the choice experiment, ant colonies were presented with one of three food pairings: (1) p42:c32 with p19:c57, (2) p19:c57 with p54:c18, and (3) p33:c43 with p54:c18. Colonies were provided with fresh food (1 cm³ cubes, centered in a small plastic weighing dish), every day, for five weeks. Any food remaining after 24 h was removed, dried for 48 h at 40 °C, and weighed. The daily amount of food collected was determined by subtracting the weight of remaining dried food from the food's initial dry weight. Food's initial dry