Research Vision Rebecca Clark

Physiology and Ecology of Nutrition in Insects

The ways in which animals obtain and allocate nutrients affects how they evolve, and how they interact with and influence their environment. I study how nutrition and behavior drive function and growth in complex social groups like leafcutter ant colonies, and how nutrient regulation affects growth and physiological allocation in individual insects such as crickets. My research thus links individual behavioral decisions, like foraging and feeding, to physiological processes such as nutrient allocation towards growth, maintenance, and preproduction. It also provides new insights into how mechanisms regulating growth, like nutrient limitation, function in complex adaptive systems.

Three nutrients are known / hypothesized to be drivers of animal growth: the macronutrients protein and carbohydrate; and the element Phosphorus. Parallel approaches from animal nutrition and freshwater ecology show that organisms are sensitive to both the absolute and relative availability of these nutrients; **my work is a pioneering effort to ascertain how interactive effects of nutrient amounts and ratios affect physiology and growth in terrestrial animals**, questions with both health-related and evolutionary-ecological implications. Because I work with insects, which are widespread, easy to care for, and fast-growing, this work is particularly well-suited for the development of student (undergraduate and Master's) research projects.

Nutrition underlies a life-history trade-off in crickets

Studies of physiological mechanisms generating life-history trade-offs have historically emphasized the role of food in terms of energetics (calories), not quality (nutrient amounts and ratios). However, different calorie sources, especially protein (p) and carbohydrate (c), have specific and different functional roles within organisms. Therefore, animals that have evolved different life histories, like dispersal vs. early reproduction, are likely to have different nutritional requirements and seek out different food sources. Wing-dimorphic crickets are a classic model system where females directly trade off allocation to flight vs. reproduction in early adulthood: long-winged (LW) females have metabolically active flight muscle, and postpone egg production, while short-winged (SW) females cannot fly, but begin reproducing earlier (Fig. 1, below). These differences are accompanied by large, robust changes in lipid synthesis and allocation to stores of flight fuel vs. egg precursors in the ovaries. I showed that specific nutrient regulation strategies also underlie this life-history trade-off: heightened selectivity by LW crickets when foods have an imbalanced p:c ratio, versus insensitivity to nutrient balance by SW crickets (Clark et al. 2013, Clark et al. in revision).

I recently expanded this work to an array of 13 diets with different relative and absolute amounts of p and c, to assess effects of nutrient concentration and p:c ratio on: feeding strategies, allocation, respiratory metabolism, activity patterns, and lifetime fitness (Clark et al, three MS in prep). This work expands our understanding of organismal nutrition and should also inform studies of bottom-up regulation of population dynamics. Ongoing efforts will tie nutrient regulation to effects on the level of intermediary metabolism, involving tools ranging from flow-through respirometry measurements, to radiotracers and analytical chemistry experiments. Collectively, these measures will permit the development of a nutritionally-explicit model of resource allocation.

Future Directions: field work and nutritional constraints on development. Crickets are omnivorous and widespread in North America. They are a key food source for higher trophic levels, and excellent for lab and field experiments on questions about nutrition, development, and population dynamics. Feeding strategies and physiological responses observed *in the laboratory* suggest the morphs have specific nutrient priorities in the wild associated with the life-history differences; this needs to be tested through population surveys and field experiments. For instance, I had an undergraduate spearhead a project to collect wild crickets and determine what they eat through gut content analysis and a simple feeding assay. I seek to engage undergraduates for such projects with locally-available cricket species as well as with lab-selected populations, and will also encourage students to pursue related questions with other local invertebrate species of interest.

Nutrition and behavior shape colony growth in ants

Eusocial groups solve many of the same organizational and developmental challenges as individual organisms; they sustain predictable growth trajectories, while coping with variation in nutrient quality and amounts. I use whole-colony manipulations of diet quality and quantity, to assess effects on survival, behavior, and colony growth / composition.

Characterizing the growth of a complex tri-trophic system. Leafcutter ants have evolved an obligate, symbiotic, nutritional relationship with a fungus - their sole food source. Workers collectively balance effort between supplying the fungus with leaves, and feeding fungus to developing brood, as the colony grows. In newly-formed colonies of the North American desert leafcutter ant *Acromyrmex versicolor*, I found that the within-colony trophic relationship between ants and fungus is dynamic and colony size-dependent even under abundant leaf availability, causing a previously unidentified selective bottleneck during colony development (Clark and Fewell 2013; Kang et al 2011).

How nutrient limitation shapes a terrestrial trophic system. Leading hypotheses suggest ant foraging decisions result from combined, interacting factors, including leaf chemistry and the fungus garden's nutritional state; factors impossible to disentangle in the field. I developed lab procedures to demonstrate, via p, c and Phosphorus supplementation, that the balance of all three nutrients affects foraging rates, fungus performance (growth), and the colony's ant population growth (MS in prep). While these experiments have been groundbreaking, particularly for demonstrating how phosphorus limitation interacts with p and c limitation, we are still in the early stages of understanding how nutrient regulation in colonies relates to nutritional conditions in the wild, which means multiple avenues of lab- and field-based inquiry need further exploration.

Behavioral regulation is a critical component in eusocial colonies; I developed scan-sampling techniques to measure how individual workers and the collective colony respond to changing task demand and resource availability. These techniques enabled two undergraduates to conduct independent honors theses, on the ant colony's division of labor during colony development, and in the context of worker size variation. Many details of colony behavior remain only poorly characterized, so expansion of this work to other ant and insect species, and across ecosystem types, will help test the generality of the growth constraints found here, and indicate more generally how nutrition affects insect growth in terrestrial ecosystems.

A synthesis of studies of cooperation. Groups of colony-founding ant queens, such as those formed by the desert seed-harvester *Pogonomyrmex californicus*, are model systems for studying the evolution of cooperation. For example, in comparing pairs of normally-cooperative vs. non-cooperative seed-harvester queens, I showed how coordination and social escalation contribute to queen and colony survival (Clark and Fewell 2014). Interestingly, harvester ant populations with queens that use these alternate strategies are next to each other, in Pine Valley versus the area surrounding Lake Henshaw. In addition to continuing studies with *P. californicus*, I seek to connect studies of cooperation in foundress associations to current knowledge of survival /growth of leafcutter ant colonies. Ant-fungus dynamics observed in young colonies occur in the context of unexplored geographic variation in colony-founding strategy in leafcutter queens (*A. versicolor*): in some areas, they begin nests alone, while in others, they start nests in groups (polygyny; Fig. 2 below). Patterns of geographic variation in colony-founding differ between species (and are poorly-studied in two cases, including *A. versicolor*). Many hypotheses about the evolution of polygyny also remain untested. For the leafcutters, initial stages will involve mapping the extent of polygyny across the species range (western Texas to California) via microsatellites/mitochondrial sequences, correlating it with environmental characteristics, and examining how polygyny affects fitness and function (e.g. survival, growth, worker polymorphism) in young colonies.

Organisms manage to thrive in environments of overabundance and scarcity, thanks to sophisticated behavioral and physiological control mechanisms. The insect systems I study, and approaches I use, show promise for determining how multiple, interacting nutrients affect the evolved control systems used by both individual and social organisms. The methods used within these systems can – and should - be readily applied to other biological contexts as well.

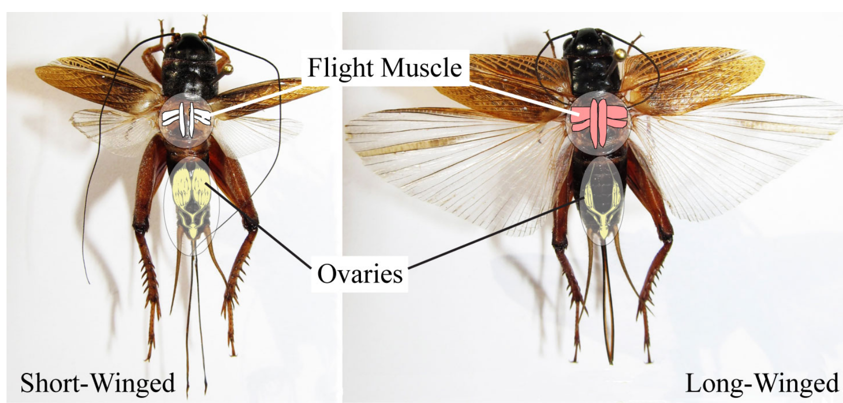

Fig. 1. Schematic illustration of the life-history trade-off during early adulthood in *Gryllus firmus* females: at Day 5, short-winged females have histolyzed flight muscles, enlarged ovaries, and cannot fly. Long-winged females have pink, metabolically active flight muscles and can fly, but greatly reduced ovaries. Shifts in both lipid and protein metabolism accompany these differences, but the role of nutrient inputs is not as well understood.

Fig. 2. Three *Acromyrmex versicolor* leafcutter queens from Arizona cooperate and coordinate leaf preparation while tending a new fungus garden. The geographic extent of this cooperative strategy is unknown, and the implications of such cooperation for colony function are poorly understood.