Metabolic Congruence or Divergence in Ant Gut Bacteria?

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It has now accepted that most animals should not be thought of as simply made of eukaryotic cells, but have a physiologically significant bacterial component. For example, humans are coated by a protective layer of commensal skin bacteria from birth, and have a large population of gut microflora that are associated with variations in body weight and general health. Together the eukaryotic and prokaryotic organism is termed a *holobiont*. However, rarely has the microbial component of the holobiont been factored into questions regarding their contribution to the evolutionary history of the host.

Our overall project is centered around the question of "how has the microbial component to the *Cephalotes* ant holobiont system contributed to ant evolution?" 117 different species of *Cephalotes* ants are found in neotropical areas, such as the Caribbean, American Southwest, and South America. *Cephalotes* are noteworthy for being herbivorous tree-dwelling species, eating diets primarily of pollen, sap, and foraging for feces and carrion. *Cephalotes* have a carbohydrate-rich and nitrogen-poor diet, which is nutritionally problematic for the ant. In addition, *Cephalotes* are unique among ants because different species share a remarkably similar community of gut bacteria. In particular for this project we are looking at *Pseudomonadceae* family of bacterial symbionts.. Preliminary tests have shown that these bacteria gain energy primarily from degradation of amino acids. As amino acid are an important nitrogen source for ants why would a gut symbiont seem to be "stealing" nitrogen from its host? Why would these ants and bacteria evolve together over millions of years if bacterial gut symbionts may be hurting the ant?

In my daily work, I am performing metabolic assays on 31 *Pseudomonadaceae* isolated from 12 species of *Cephalotes* ants. I use 96-well plates in addition to single-tube media to quickly assay substrate utilization. Potential organic acid excretion is measured via gas chromatography, and specific amino acids the bacteria are capable of consuming and excreting is quantified via high-performance liquid chromatography.

Preliminary results show that all 31 *Pseudomonads* that I am working eat amino acids and organic acids like citrate and acetate. This is remarkable consistency of function across 12 divergent species of ants! We are also seeing that they produce certain organic acids like butyrate, and are continuing HPLC-based tests to check for production of amino acids. We hypothesize that the bacteria we are studying are co-metabolizing food with the ant host, degrading non-essential organic and amino acids and producing ones that the ant needs for growth and respiration. This would highlight a strong evolutionary relationship between *Cephalotes* and their *Pseudomonadaceae* symbionts.

Personally, I feel that this research has helped me further round out skills I have learned at Calvin. After this summer, I am much more familiar with microbiology techniques. I've had the opportunity to learn theory in application of analytical chemistry, and developed skills in using advanced instruments, something I never thought I would have to do. I've also been able to strengthen concepts to do with evolutionary relationships between organisms, and have learned much about new theories in biology such as the holobiont theory. As always, research is problematic, and patience is necessary.