Using Stable Isotope Analysis to Study the Exchange of Nutrients between Myrmecophytes and their Mutualistic Ants in an Amazon Rain Forest

Faculty Advisor: John Bevington, Professor
Department of Biological Sciences

SOAR Project Proposal
Summer, 2017

Students: Kyle Froehlich stkrf06@moravian.edu Major: Environmental Science
Danniel Gerrity gerrityd@ moravian.edu Major: Health Sciences

Project Timetable: Duration: 6 weeks 15 May – 26 June 2017

Due to logistics in Peru and constraints with exporting research samples, our project will begin mid May. Week 1: student literature searches, Week 2: depart for Peru, travel to Cocha Cashu, Week 3: fieldwork and collecting in the forest, Week 4: complete fieldwork, return to Cusco, Week 5: organize samples, review field notes, return home,Week 6: data analysis, write reports, arrange for stable isotope analysis

Project Summary:

Myrmecophytes are plants that form symbiotic associations with ants. This proposal is a request for financial support to allow two SOAR students to accompany me this spring into the Peruvian Amazon to initiate a research project to study nutrient exchange between myrmecophytes and their mutualistic ants. The project will be undertaken at the Cocha Cashu Biological Station, a remote field station in Manu Park. We will travel to Cocha Cashu this spring to collect specimens of species of myrmecophytes and their associated ants, and to make observations relevant to the natural history of these mutualisms. The species we plan to study are listed in Table 1. The samples we collect will be used to detect nutrient exchange between the plants and their ants using a method called stable isotope analysis. This utilizes a specialized instrument, an isotope-ratio mass spectrometer. This is an expensive instrument and we do not have one. However, it is possible to send samples for analysis to laboratory facilities at large universities, or to industrial laboratories for analysis at reasonable costs on a per sample basis. This is what we plan to do. On return from Peru it will be 1-2 months before we receive export permits for our specimens; so we request only 6 weeks of SOAR support.

The principle behind stable isotope analysis:

Many elements exist as two or more stable (non-radioactive) isotopes. Nitrogen, for example exists as ^{14}N and ^{15}N and carbon as ^{12}C and ^{13}C. The ratio of the heavier to the lighter isotope, or the delta value (δX), represents the deviation per mil (o/oo) from an arbitrarily designated standard. The isotopic carbon composition of animal tissues closely reflects the isotopic composition of the food eaten. The $\delta^{15}N$ of plant tissues typically reflects the $\delta^{15}N$ of the plant's nitrogen source. Stable isotopes have been widely used to trace the flow of nutrients

between organisms and their movement in food webs. In these experiments we will be looking for nitrogen enrichment in plant tissues of the myrmecophytes such that the nitrogen isotopic composition is skewed toward the $\delta^{15}N$ values of mutualistic ants living in the domatia of the plants.

Table 1. Myrmecophyte plants and their associated ants reported at Cocha Cashu Biological Station in Manu Park, Peru

Family	Genus	Species	Associated Ants
Boraginaceae	*Cordia*	*nodosa*	*Myrmelachista spp.*
Chrysobalancaceae	*Hirtella*	*racemosa*	*Allomerus octoarticulata*
Caesalpiniaceae	*Tachigali*	*myrmecophila*	*Pseudomyrmex concolor*
Melastomataceae	*Maieta*	*guianensis*	*Pheidole minutula* (?)
Melastomataceae	*Tococa*	*guianensis*	*Myrmelachista spp.*
Polygonaceae	*Triplaris* *	*americana*	*Pseudomyrmex* spp.
Rubiaceae	*Duroia*	*hirsuta*	*Myrmelachista spp.*

* We are especially interested in *Triplaris americana*, the fire-ant tree. The *Pseudomyrmex* ants that inhabit it are very aggressive and particularly pugnacious.

Roles and Responsibilities of the Students:

Due of the amount of fieldwork, two students will be required to complete the work at Cocha Cashu. Both will be actively engaged. Their work will involve a variety of tasks including locating and identifying myrmecophytes, confirming that they have been colonized by ants, and photographing the plants and their associated ants. They will assist with collecting samples to be used later for stable isotope analysis. Plant materials will be collected in plastic ZipLock® bags, transferred to plant presses, and blotters and driers changed daily. Ants will be collected with forceps and pooters, then transferred to screw cap glass vials. Students will be responsible for collecting the relevant data about the plants and ants and for maintaining a daily data log. In short, the students will be engaged in all aspects of the project. Because of the amount of fieldwork to be done at Cocha Cashu, half of the species in Table 1 will be the responsibility of one student and half will be tasked to the other student. Prior to departure for Peru both students will conduct online literature searches to familiarize themselves with the primary literature on the natural history of these ant-plant mutualisms. At Cocha Cashu I will mentor both students in the field.

Roles and Responsibilities of the Faculty Member:

The faculty member will be responsible for travel and lodging arrangements, logistics in the field, assembling field gear and supplies, and identification manuals.

Background for the Proposal:

For several years I have been studying anti-herbivore defenses in *Cecropia* trees. In *Cecropia* it is known that there is a substantial contribution of nitrogen to the trees' nutrient budget from its *Azteca* ants. If this phenomenon can be

demonstrated in mutualistic ants of other myrmecophytes (unrelated to *Cecropia*), it would suggest it that mutualistic ants may play a larger role in the nutrient cycling of the forest as a whole. We will include *Cecropia* here for comparative purposes. Recent publications supported by SOAR awards to my students can be found here:

http://onlinelibrary.wiley.com/doi/10.1111/btp.12163/full
http://www.mdpi.com/1420-3049/19/9/14484/htm

There may be more than 250 Amazonian plants that form associations with ants. Many of these are mutualistic, meaning that both the ants and the plants derive adaptive advantages from their interactions with each other. Structural features of the plants encourage ant recruitment. Plants provide domatia in which the ants build nests (e.g. hollow internodes, swollen petioles, or leaves). Many myrmecophytes provide their ants with food bodies that the ants harvest and transport into their nests. These are variously called Müllerian bodies, pearl bodies, or Beltian bodies. To encourage the ants to remain on the plant, some myrmecophytes also produce extrafloral nectaries. In return for these benefits, ants defend their plants from herbivores and prune encroaching vines that grow over the host's leaves. For some myrmecophytes an added benefit is that nutrients flow into the plant simply because of the ants' presence. In effect this means that the ants "feed their plants," i.e. they fertilize them. The basis for this seems to be that mutualistic ants are not particularly tidy housekeepers in their domatia. Ant nests often contain debris, frass, and insect carcasses from their prey captured during foraging excursions. Microbial decomposition of this organic material releases nutrients that become available to the host plant by absorption through the walls of domatia. The magnitude of this effect is probably related to the size of the ant colony with larger ant colonies providing more nutrients than smaller ones. One study has shown that the ants in *Cecropia peltata* trees supply >90% of the trees' nitrogen from the debris they leave in the hollow stems. In effect, the ants provide not only bodyguard services against herbivores but also a substantial nutritional benefit in the form of nitrogen fertilization. During my fieldwork in the Madre de Dios of Peru I began to wonder if other myrmecophytes exchange nutrients with their ants. If so, nutrient exchange in mutualistic ant-plant relationships may actually be a two-way street with nutrients moving from the plant to its ants via food bodies from the plant, and nutrients flowing back to the plant (inadvertently) from microbial action on the frass and detritus left in the ants' domatia.

There is an interesting twist to nutrient flow in some ant-plants. Some myrmecophytes have ants that tend trophiobionts (coccoid or pseudococcoid insects, i.e. mealy bugs), as we would tend cattle. Coccoids use piercing mouthparts to consume plant juices; the ants then drink the "honey dew" exuded by the coccids from their anuses. The ants may also eat coccids. Hence, nutrient flux within the mutualism may involve more than just the plants and the ants. It may be tripartite.

Why this work is important: This project is a pilot study to determine if nutrient exchange is found widely between myrmecophytes and their ants, or if it is simply an isolated phenomenon in a few species. Widespread nutrient exchange would suggest important implications for the role of myrmecophytes in tropical forests.

SOAR Project Proposal
Summer 2017
Student Statement of Purpose

Project: **Using Stable Isotope Analysis to Study the Exchange of Nutrients between Myrmecophytes and their Mutualistic Ants in an Amazon Rain Forest**

Student: Kyle Froehlich
Major: Environmental Science
Graduation: May 2019
Faculty Mentor: Dr. John Bevington
Campus Housing: No

As a rising junior, I can still remember the impact Botany class had on my interests and direction of life. I applied to Moravian College with the intention of majoring in physics and engineering. However, my objectives dramatically changed, as I seemed to detest the required mathematical skills needed for physics. Environmental science was always on the back burner for me as nature contains a large and important part of my life. Consequently, when I took Dr. Bevington's Botany class, I exploded into curiosity. I researched topics outside of the syllabus and developed an thirst for more knowledge. It wasn't long until my inquisitiveness lead me to invested in a used microscope, some dye, and lots of microscope slides. Through this equipment, I advanced to many new topics I hadn't been exposed to yet; this led me to experience the scientific process of observing, hypothesizing, and analyzing. Now, I am studying entophytes that I found in a local weed called *Pilea pumila*. This discovery has opened up many new doors, which I never saw before. Moreover, I want to continue down this captivating road I'm on. This opportunity to study in the Amazon Rainforest side by side with a professor with whom I have developed a strong bond is an opportunity of a lifetime. Learning and, most importantly, experiencing professional research methods and processes allows me to grow to where I can conduct professional research of my own. Additionally, experiencing Peru and the Amazon Basin would permit me to better learn about and understand other cultures in a way that books cannot provide. This will be an awakening in which I develop a deeper understanding for the rainforest and its diversity.

Over the past couple of semesters, I have informed Dr. Bevington concerning my interest in researching, especially in the Amazon. His focus of study intrigues me and also has merit to my own endophytic research. The *Cercropia* tree and its mutualistic ants are fascinating to me because of their interdependence. The ants rely on the tree for a home while the tree relies on the ants' for nitrogen uptake. Additionally, ants can act as bodyguards for the tree in many extraordinary ways.

However, as important as these ants are, there are a few species of *Cercropia* that do not support mutualistic ants. So, what separates these trees from the others?

This project will provide a better understanding of the mutualistic relationship between the ants and the trees by allowing us to form connections. This knowledge could be used to better understand other mutualistic relationships in nature. Lastly, I would like to point out that Dr. Bevington will benefit from my help just as I will benefit from him. My inquisitiveness will challenge and create new pathways for Dr. Bevington's research. I truly am interested in Dr. Bevington's research and hope this plan becomes a real opportunity.

SOAR Project Proposal
Summer 2017
Student Statement of Purpose

Project: Using Stable Isotope Analysis to Study the Exchange of Nutrients between Myrmecophytes and their Mutualistic Ants in an Amazon Rain Forest

Student: Daniel Gerrity
Major: Health Sciences
Graduation: 2019
Faculty Mentor: Dr. John Bevington
Campus Housing: No

The project proposed by Dr. Bevington strives to study at least seven specific myrmecophytes and ants with which they have a mutualistic relationships. The scope of the project includes collecting scientific samples and using stable isotope analysis to examine the flow of nutrients in ant-plant mutualisms. We will travel to Cocha Cashu Biological Station in Peru where Dr. Bevington has located some of the species we wish to study. Once there we will collect samples and experiment for three purposes.

Contribution to the discipline:

1. To discover the prevalence of nutrient exchange found in mutualisms between myrmecophytes and their ants.
2. To study the evolutionary question of nutrient flow stabilizing mutualism and preventing the relationship from becoming parasitic.
3. To study the relationship between the nitrogen exchange and the nutrient availability of the soil of the Amazon tropical forest at this site. This is important because the soils of the Amazon are generally nutrient poor. If the nitrogen flow between myrmecophytes and their mutualistic ants is as prevalent as Dr. Bevington's suspects, our project could identify one of the key elements that stabilizes the ecosystem.

   Our work would not only collect valuable data on an interesting evolutionary phenomenon, but it could uncover one of the key links in an entire ecosystem's sustainability.

Benefit to me as a student:

The project proposed by Dr. Bevington will give me experience in experimental processes and application of results that I simply cannot gain any other way before graduation. Not only is the subject of the experiments of utmost interest to me as a student-scientist, but it could clarify a part of what makes the most important ecosystem on our planet thrive. During the project I

will receive first-hand experience in the art of data gathering, scientific analysis, experimental processes, and learn how to present work to the academic community. My goal when I left the Navy to study at Moravian College was to get an undergraduate degree necessary to becoming a Physician Assistant so I could expand upon what I learned as a Surgical Technologist. During my time here, however, I have been enlightened to the many ways an educated person can shape our world for good, and I have been considering ecological science as a possible avenue for my efforts. This SOAR project would give me an opportunity to see the exact type of work I have been considering. Gaining experience in the Scientific method, experimentation, and the process in which experiments are presented and discussed amongst colleges is an opportunity that will help me to be prepared for professional scientific work no matter which discipline I choose.

Using Stable Isotope Analysis to Analyze the Exchange of Nutrients between Myrmecophytes and their Mutualistic Ants in an Amazon Rain Forest

SOAR Travel Grant Proposal, 2017

Requested amount: $2000 ($1000 for each of two students)

Faculty Advisor: John Bevington, Professor

Department of Biological Sciences

Students:

Kyle Froehlich	Major:	Environmental Science
	Class rank:	Sophomore
	Graduation:	2019
Danniel Gerrity	Major:	Health Sciences
	Class rank:	Sophomore
	Graduation:	2019

Basis for the Request:

In the spring of 2017 Kyle Froehlich and Dan Gerrity will travel with Dr. Bevington to Peru to pursue the research project titled above. A project summary, the research goals, and the roles and responsibilities of the students and the faculty member are detailed in an attached SOAR Summer Research Proposal with the same title. Briefly, the goal of the project is to identify and collect leaf samples of myrmecophytes and their associated ants. These samples will be used in a pilot study to examine nutrient exchange between the plant hosts and their resident, mutualistic ants. The fieldwork will be done at Cocha Cashu Biological Station in the lowland tropical rainforest of Manu Park, Peru.

The principal expense of this undertaking for the students is their travel costs, and this proposal is a request for funds to support some of the travel expenses incurred by the students. These include round trip international airfare, Newark/Lima/Newark (ca. $825), and one in-country flight into the Andes from the Pacific coast of Peru, Lima/Cusco/Lima ($250). These airfares will consume most all of the students' requested travel awards. From Cusco it will take 3 days for us to reach Cocha Cashu Biological Station deep in the lowland tropical forests of Manu

Park in the Madre de Dios of Peru. We will travel east by vehicle from Cusco through the high Andes. From the crest of the Andes near Paucartambo the road descends the eastern slopes into cloud forests. About half way down the mountains the road reaches Atalaya, a small village on the Alto Madre de Dios River where we transfer to a long canoe and continue the descent out of the Andes into the Amazonian lowland forests near the confluence of the Alto Madre and the Manu Rivers. From there it is 9-hr boat ride up the Manu River to the research station. Additional expenses for the students will include station fees and meals for our 7-day stay at Cocha Cashu (ca. $280), lodging in Lima, Cusco, in the cloud forest and at Boca Manu at the base of the Andes (ca. $500, 10 nights lodging x ca. $50/night, double occupancy). Pro rata shares of the costs for vehicle and boat transport including driver and cook (TBD).

Anticipated Outcome:

After we have been able to analyze the stable isotope ratios of our samples, we would hope that the results would be significant and that one or more of the students could present the results at a professional meeting. This might be a local meeting such as the annual spring meeting of the Lehigh Valley Society of Ecology and Evolution. However, if the data were to be good enough I would hope that one of the students could present at a national meeting (e.g. ESA, Ecological Society of America), or maybe even an international meeting (ATBC, Association for Tropical Biology and Conservation). That decision would be made at a later date.

I anticipate that interesting results from student participation in this project might promote involvement of other students over the next few summers to gather more material samples and field data at Cocha Cashu. And perhaps it could be used to attract funding for student travel from an outside source. We were able to do this once 4 years ago.