Do Leaf-Cutter Ants Attack Myrmecophytes That Support Colonies of Mutualistic Ants?

SOAR Project Proposal
Summer, 2017

Faculty Advisor: John Bevington, Professor
Department of Biological Sciences

Student: Luke Peterson stlip01@moravian.edu Major: Music

Project Timetable: Duration: 6 weeks 15 May – 26 June 2017 3

Our project will begin in mid May.

Week 1: student literature searches,
Week 2: depart for Peru, travel to Cocha Cashu,
Week 3: fieldwork, locate leaf-cutter nest(s) and myrmecophytes, run pick-up assays, behavioral observations of mutualistic ants, collect ants for later species identification, photo documentation of all ant and plant species to be utilized in the project
Week 4: complete fieldwork, return to Cusco,
Week 5: organize and review field notes, return home,
Week 6: data analysis, write reports, arrange for stable isotope analysis

Project Summary:

This spring Luke Peterson along with two other students, Kyle Froehlich and Dan Gerrity, will travel with Dr. Bevington to the Cocha Cashu Biological Station in Manu Park in the Peruvian Amazon. This proposal is submitted to allow Luke to pursue a SOAR study of the interactions between a leaf-cutter ant (*Atta* spp) and myrmecophyte plants. Leaf-cutters are important herbivores in the forest, and myrmecophytes are plants that are typically defended from herbivores by mutualistic ants that live in their myrmecophyte hosts. The mutualistic ants act like bodyguards. So mutualistic ants are different from the leaf-cutters. We posit that the mutualistic ants in the myrmecophytes should be able to repel leaf-cutting ants encroaching on their host tree, and that this effect should be stronger with the more aggressive mutualistic ants than with timid or shy ants. Similarly, we suggest that myrmecophytes with larger colonies of mutualistic ants should be better defended than those with small colonies of mutualistic ants.

Background for the Proposal:

This project will involve two kinds of ants. We are interested in the interactions between these two ants because they assume very different ecological roles in the rainforest ecosystem. Leaf-cutter ants, as their name implies, harvest small pieces of leaves and transport them along their foraging trails to their nests.

Leaf-cutters belong to two genera of the New World Attine ants, *Acromyrmex* (24 species) and *Atta* (15 species). Both genera harvest leaf tissue to grow fungi in their nests for food. They are in effect "fungus farmers." Most of their leaf harvesting is done in the forest canopy. From their nests they travel along their foraging trails, ascend tall trees, cut small pieces of leaves, and then return to the forest floor and transport the leaf fragments back to their nest. Some species are very active at night. For a large nest, foraging trails can exceed 100 meters or more in length, and they are frequently branched. Leaf-cutters are ecologically important because they harvest large quantities of leaf biomass, and for this reason they are the most important herbivores in the forest. Their harvesting potential is impressive. For example, two studies in Panama showed that *Atta colombia* harvests between 85 - 470 kg dry weight of plant biomass from the forest over one year per colony. This represented an approximate leaf area of 835 - 4550 square meters per year per colony! Moreover, in a lowland Neotropical forest there may be several leaf-cutter nests in a single hectare. Colonies of some *Atta* species may contain up to 8 million ants with the collective biomass of an adult cow. They can be thought of as "ecosystem engineers" because directly or indirectly they alter the availability of resources to other organisms in the forest. This contributes to both the spatial and temporal heterogeneity of resources. Therefore, they contribute to the creation of niches for other species and thus to the distribution of other species.

Mutualistic ants are known to inhabit a number of myrmecophyte hosts (ant-plants). These ants live in domatia (hollow stem internodes, swollen leaf chambers and petioles) on a variety of host plants in a number of different plant families. Most of these mutualistic ants defend their host plants from herbivores by attacking them when they attempt to encroach onto their myrmecophyte hosts. At Cocha Cashu seven myrmecophytes harboring mutualistic ants have been reported (Table 1). We hope to locate most if not all of these for our study. There may be others as well.

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.*

Experimental Strategy:

Our first goal will be to locate active colonies of leaf-cutter ants in the vicinity of the field station. The station Director Cesar Flores has advised us that there are leaf-cutter nests near the station. However, we do not know how many. It would be

ideal to find more than one nest to work with. We will follow foraging trails away from the nest(s) to determine if the ants' trails go near any myrmecophytes, and if they are harvesting leaves from them. Next we will try to assess when foraging activity along the trails is greatest. Then insofar as possible we will try to run our experiments at these times. Our strategy will follow the protocol outlined here:

1. *First, we want to determine if leaves of the myrmecophytes are acceptable to the leaf-cutter ants in the absence of any mutualistic ants, i.e. are they "palatable?"* We will use pick-up assays to determine this. Once we have located the myrmecophytes we will harvest leaves, collecting both young, immature leaves and mature, fully-expanded leaves. It is known that most herbivore damage to leaves occurs on young leaves before they reach full size; however, in previous work I have found that for some species of *Cecropia*, leaf-cutters may prefer mature leaves instead. Leaves will be transported back to the station where we will cut small circular pieces with a cork borer or paper punch (4-7 mm diam) and small rectangles (2 x 4 cm). These will be transported to a leaf-cutter foraging trail on moist filter paper in plastic Petri dishes. Using forceps the samples will be displayed along the trail at regular intervals. Using stopwatches the number and kinds of leaf samples taken by the ants over a 30-minute interval will be recorded and compared to the number of oat flakes presented in a similar fashion as controls. Leaf-cutters love oat flakes and accept them readily. Data will be expressed as a ratio of leaf fragments taken to oat flakes taken/30 minutes and subjected to statistical analysis. This should give us an idea if leaf-cutters will accept myrmecophyte leaf tissue in the absence of harassment by mutualistic, defensive ants living on the host plant.

2. *Will the mutualistic ants that inhabit host myrmecophytes attack leaf-cutters?* The answer to this question will tell us whether the resident mutualistic ants are effective bodyguards for their host myrmecophytes against leaf-cutter foragers. We suspect the answer here may be different for the different ants in Table 1. We would do this experiment in the following way. The leaf-cutter foragers would be captured on their trails using small jeweler's forceps and pooters (aspirators for sucking small insects through a tube into a bottle). Ideally we would be able to locate myrmecophyte(s) near a leaf-cutter nest; this would simplify the logistics of transporting leaf-cutter foragers through the forest from their trails to a myrmecophyte with a colony of mutualistic ants. The leaf-cutters would then be gently transferred onto leaves of a myrmecophyte near the mutualistic ants. Behavior of the mutualistic ants would be followed over time. In particular, we will look for behavioral evidence that might indicate that mutualistic ants recruit their nestmates when they have detected the intruding leaf-cutter foragers.

3. *Finally, will leaf-cutters display harvesting behavior on myrmecophytes if we prevent the mutualistic ants from harassing them?* We plan to do this by protecting leaf-cutters with Tanglefoot to isolate some leaves from others.

SOAR Project Proposal
Summer 2017
Student Statement of Purpose

Project: Do Leaf-Cutter Ants Attack Myrmecophytes That Support Colonies of Mutualistic Ants?

Student: Luke Peterson
Major: Music
Graduation: 2019
Faculty Mentor: Dr. John Bevington
Campus Housing: Yes

Never did I think my dream would come true for the opportunity to travel to the Amazon Rainforest and immerse myself in its culture and knowledge. Even though my major is music composition my first love has been the natural outdoors, even as a kid I would ask questions and then go outside to research and find the answers. I have done a good deal of work strengthening myself musically, composing for the marching band and Moravian choir but I have not worked as hard on my minor in Environmental science.

I am intrigued with the mutualistic ants that defend myrmecophytes like the *Cecropia* trees against herbivores, and also the leaf-cutter ants that are themselves herbivores. This SOAR project will give me the opportunity to see if leaf-cutter ants will attack myrmecophytes even though the plants have mutualistic bodyguard ants on them, and I can run experiments to see if leaf-cutter ants will pick up pieces of myrmecophyte leaf tissue that I put along their foraging trails. My project will run parallel to another project by Dan and Kyle. They will be collecting samples for stable isotope analysis in a separate study to see if there is nutrient exchange between mutualistic ants and their host myrmecophytes. I will be asking a different question. I want to know if leaf-cutter ants can attack myrmecophytes and harvest leaf tissue. Having a once in a life time opportunity to gain knowledge as an environmental scientist as well as travel to the Amazon will give me an excellent source of fieldwork, time in the lab and library, and an opportunity to work effectively with other people. I will go out into the rainforest and find different species of myrmecophytes test to see if the leaf-cutter ants can attack them. This will build my strength in collaborating with other people as well as individually building my expertise in something that I research by myself. Researching questions to find answers will definitely give me a sense of pride and accomplishment. Being a part of a SOAR project will push me to keep asking questions and give me the experience as an environmental scientist to better prepare me for the outside world.

Do Leaf-Cutter Ants Attack Myrmecophytes That Support Colonies of Mutualistic Ants?

SOAR Travel Grant Proposal, 2017

Requested amount: $1000

Faculty Advisor: John Bevington, Professor
Department of Biological Sciences

Student: Luke Peterson

Major:	Music
Class rank:	Sophomore
Graduation:	2019

Basis for the Request:

In the spring of 2017 Luke Peterson will travel with Dr. Bevington to Peru to pursue the research project titled above. A project summary, the research goals, experimental strategy, and the roles and responsibilities of the student and the faculty member are detailed in an attached SOAR Summer Research Proposal with the same title. Briefly, leaf-cutter ants are important herbivores in the rainforests of the Neotropics. These ants harvest leaves from canopy trees and transport them down to the forest floor then to their subterranean nests where they use them to grow a fungus. The fungus is the sole source of food for the ants. These ants are ecologically very important because they attack many species of trees. However, some trees in these forests have developed mutualistic associations with other species of ants. They provide domatia and special food bodies to recruit their ants. These ant-plants, or myrmecophytes as they are called, benefit from their mutualistic ants because the ants act as bodyguards to deter herbivores. Little is known about how susceptible myrmecophytes are to leaf-cutter ant attack. This is the focus of Luke's SOAR project. The fieldwork will be done at Cocha Cashu Biological Station in the lowland tropical rainforest of Manu Park, Peru.

Luke and Dr. Bevington will travel to Cocha Cashu with two other SOAR students, Kyle Froehlich and Dan Gerrity. Kyle and Dan will also be pursuing a project using myrmecophytes and mutualistic ants, but not leaf-cutter ants. The question they are asking is entirely different and their methods are different as well. They will collect specimens of myrmecophytes and their mutualistic ants with the goal of determining if there is nutrient exchange between the ants and their host plants.

Because of the remote location of Cocha Cashu the major costs for this project for Luke will be his travel expenses. This proposal requests funds to defer some but not all of Luke's travel expenses. These include round trip international airfare, Newark/Lima (approx. $825), and one round trip in-country flight into the Andes, Lima/Cusco (approx. $250). Airfares will consume all of the students' requested travel award. There will be appreciable addition expenses for each of us. From Cusco it takes 3 days for us to reach Cocha Cashu Biological Station deep in the lowland forests of Manu. From Cusco we will travel east by vehicle through the Andes. At the eastern crest of the Andes near Paucartambo the road descends down the eastern slopes through cloud forests. Half way down the mountains the road reaches the small village Atalaya on the Alto Madre de Dios River. There we transfer to a long canoe and descend out of the Andes and into the Amazonian lowland forest to the confluence of the Alto Madre de Dios and the Manu Rivers. It is approximately a 9-hr boat ride up the Manu River to the research station. Shared costs for vehicle and boat transport including driver, cook, and motorman (TBD) are pro rata. Additional expenses 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).

Anticipated Outcome:

Very little is known about interactions between leaf-cutter ants and the mutualistic, defensive ants that inhabit myrmecophytes. We hypothesize that mutualistic ants confer an adaptive advantage to their myrmecophyte hosts by defending them against leaf-cutters. We also think that some species of mutualistic ants may be better bodyguards than others. If Luke can locate three or four different myrmecophytes with resident mutualistic ants, he may be able to determine if they display different levels of aggression toward invading leaf-cutters on their hosts. This would be an important observation and would in principle would have evolutionary implications.