SOAR Proposal – Summer, 2013

Anti-herbivore defense against leaf-cutter ants in myrmecophyte and non-myrmecophyte *Cecropia* trees in tropical premontane forests of Manu Park, Perú

Students:  
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Sponsoring Faculty Member:  
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Start Date: 24 May 2013  
Length of Project: 4 weeks

Location: Tropical rain forest, Perú

Amount Requested: $5100

**Project Summary:** This is a request for support for two students to participate in a summer research project on anti-herbivore defense strategies in *Cecropia* trees. We will continue a study of how the trees defend themselves against leaf-cutter ants. Support is requested for 4 weeks. The proposal requests stipends and travel support in Perú for both students to travel with me in June 2013. Dormitory housing for both students is also requested for their time on campus before we leave for Perú and for the time after we return to analyze the data and write up their work. I have also requested a faculty stipend. However, I do not plan to take the stipend as remuneration. Rather, if the proposal is funded I would ask that the Business Office apply it to student travel expenses to defray their costs during our time in Perú. The students will be actively engaged in field work in Perú which will last about 2 weeks. They will help me locate leaf-cutter ant nests and the ants’ foraging trails and collect specimens of the worker and the soldier ants for taxonomic identification later. Students will also locate *Cecropia* trees near the leaf-cutter nests. Students will harvest leaf samples, conduct field experiments, collect data, and prepare voucher samples. The project will be carried out in the rainforest near Pantiacolla and leaf samples will be collected along the Río Alto Madre de Dios and in the adjacent forests along the river. From previous trips to Pantiacolla I know that there are leaf-cutter ant nests nearby. Our coordinator in Perú told me recently that these nests are still present and active.
Background for the Proposal. Insect herbivores reduce the surface area of leaves and for this reason they diminish photosynthesis. This is particularly important in tropical forests because insect diversity is so high. Insect herbivores include adult and larval forms of beetles, aphids, true bugs, and the caterpillars of moths and butterflies. Plant anti-herbivore defenses are evolutionary adaptations to herbivore pressure. Many of these defenses are chemical in nature, and they are often concentrated in the young, emerging leaves. This is because young leaves are most vulnerable to herbivore attack. They have a high water content, high levels of protein, and they are easier to chew than older leaves.

Some tropical trees have evolved mutualistic relationships with ants to defend themselves against herbivores. The ants live in the trees and harvest food bodies produced for them by the trees. As payment to the trees the ants defend the trees against herbivores, often aggressively so. Plants that harbor such mutualistic ants are called myrmecophytes (ant-plants). *Cecropia* trees are well known myrmecophytes in the tropical forests of Amazonia. Of the sixty recognized species of *Cecropia* all but seven or eight are myrmecophytes, and most are inhabited by *Azteca* ants. I am interested in the non-myrmecophyte *Cecropia*. I want to know how these *Cecropia* defend themselves against herbivores without mutualistic *Azteca* ants and whether the non-myrmecophytes evolved from myrmecophytes, or vice versa. We will be looking for five species: *C. membranacea*, *C. chlorostachya*, *C. polystachya*, *C. sciadophylla*, and *C. tacuna*. The first three are myrmecophytes, and the last two are non-myrmecophytes.

Leaf-cutter ants are important herbivores. An interesting twist to this story is the role of another group of ants. These are the leaf-cutters, *Atta* (comprised of 15 species) and *Acromyrmex* (24 species). Rather than being beneficial, mutualistic bodyguards on *Cecropia* trees these ants are important herbivores. In fact, leaf-cutters are probably the single most important herbivores in Neotropical forests. They harvest leaves from trees in the forest, carry the leaf fragments down from the canopy, and transport them along their foraging trails to their underground nests. They do not consume the leaf fragments that they harvest. Instead they use them to grow a subterranean fungus. The fungus is the ants’ sole food source; they do not eat the leaf tissues. In effect, these leaf-cutters are both herbivores and “fungus farmers.” Although the *Azteca* ants of *Cecropia* trees are an effective defense force against the leaf-cutters, we want to know how the non-myrmecophyte *Cecropia* avoid leaf-cutter attacks.

What we know about leaf-cutter preferences for *Cecropia* leaves. I have been studying the chemical basis for anti-herbivore defenses in three *Cecropia* species in Peru. One of these is the non-myrmecophyte *C. sciadophylla*. Field observations suggested that leaf-cutters apparently do not attack *C. sciadophylla* even though it lacks mutualistic *Azteca* ants. We know now that its leaves are heavily defended chemically. Experiments in the spring of 2012 in Ecuador indicated that samples of both young and mature leaves from *C. sciadophylla* were refused by leaf-cutters when presented to them along their foraging trails. However, for *C. membranacea*, a myrmecophyte, the leaf-cutters’ response was different. Samples of immature leaves were rejected as was the case with *C. sciadophylla*, but the leaf-cutters accepted approximately 50% of the mature leaf samples from *C. membranacea*. We want to continue these experiments at Pantiacolla and investigate the basis for differences in sensitivity to leaf-cutter attack between myrmecophyte and non-myrmecophyte *Cecropia*. We will try to test the hypothesis that anthocyanin pigments in young leaves of *C. sciadophylla* inhibit leaf-cutter attack. Anthocyanins in some plants have anti-fungal properties.
**Experimental Strategy.** We will assess leaf preferences of the leaf-cutters using a pick-up assay. Leaf fragments of standardized sizes from young and mature leaves will be presented to the ants as they move along their foraging trails. Times required for the ants to pick them up will be recorded with stopwatches as well as the distances the ants carry the fragments if they pick them up. In other experiments we will present the ants with larger pieces of leaves to see if the ants will cut them up and then carry them away to their nest. We plan to measure the levels of anthocyanins in alcohol extracts using a hand-held spectrophotometer and determine if pigment concentration correlates with ant behavior. I am particularly interested in the responses of leaf-cutters to leaves of myrmecophyte *Cecropia* in the absence of their *Azteca* bodyguard ants.

**Students’ Roles and Responsibilities.** SOAR students will assist with field work in the rainforest and help prepare and organize field gear before departure. When we return they will analyze data statistically and write reports. Because the Collier Hall of Science will not be available in the summer of 2013, we will not be able to analyze the leaf samples chemically. Students will have specific tasks while we are in Perú: (1) They will collect *Cecropia* leaf samples in different stages of development and prepare them for presentation to the leaf-cutter ants in pick-up assays. They will assist in locating leaf-cutter nests, collecting voucher specimens of ants for identification, and preparing leaf extracts to estimate anthocyanin levels. (2) They will also record field data (GPS readings, digital photographs, morphological characters, etc.), and they will help prepare herbarium specimens.

**Summary of Benefits.** The value to the students is that it will allow them to participate in a field research project in the most biologically diverse ecosystem on earth, one which few students have the opportunity to see. They will collect data, analyze it statistically, draw appropriate conclusions, and write a scientific report on their work. This should give them an opportunity to see how ecological adaptations may vary between related species, and I hope it will be possible for one or both students to present the work at a scientific meeting. Results from the last two SOAR research expeditions were presented at national meetings with the students as lead authors. Research from the 2010 SOAR project created the opportunity for an Honors project the following year, and I anticipate that other students may be able to participate in this manner. Finally, three of my four SOAR students who have graduated have gone on to graduate school or medical school. Their SOAR research projects were important in being accepted and in two instances for their receiving financial support.

**Budget:**

Student summer stipends. One week of work on campus followed by two weeks of field work in Perú and then another one week on campus for data analysis and paper work:

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2 \times \$300/\text{week} \times 4 \text{ weeks} = 2400
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Faculty stipend for mentoring two students: \( \$175/\text{week} \times 4 \text{ weeks} \) = 700

Travel expenses for two students for field work: \( \$1000 \times 2 \) = 2000

Total request = 5100
SOAR Student Statement of Purpose – Spring 2013

Anti-herbivore defense against leaf-cutter ants in myrmecophyte and non-myrmecophyte Cecropia trees in tropical premontane forests of Manu Park, Peru

Name: Alex Huynh
Major: Biology
Class year: 2014
Email: stavh02@moravain.edu
On campus housing request: Yes

Faculty Mentor: John Bevington
Professor of Biological Sciences
jmb@cs.moravian.edu

The SOAR project I am applying for will provide me the opportunity to work alongside Dr. John Bevington, my biology teacher and personal academic advisor, and Vincent Reed, a fellow biology major. The purpose of this specific project is to assess herbivore defense mechanisms in both myrmecophyte and non-myrmecophyte Cecropia trees and compare anthocyanin (herbivore defense chemical) levels in both young and mature leaves of each respective group of trees. We will collect data at Pontiacolla Lodge in Manu Park, Peru, ultimately observing the rate of respective leaf fragment acceptance by leaf-cutter ants (Atta spp.), an important Neotropical forest herbivore. We will conduct a pick-up assay to accomplish this. Besides data collected from the pick-up assay, we will also collect accompanying information to add to our final project reports such as digital photographs, ant nest and plant locations, voucher specimens, and anthocyanin concentration levels in the leaves.

There are two reasons behind applying to this project. The first is that this experience will give me a firsthand demonstration of all that I have been learning in my biology major classes. Being able to observe and study the topics I have only ever seen in text books will undoubtedly shed a new light on all the knowledge I’ve acquired at Moravian College, while giving me the once in a lifetime opportunity to immerse myself in the most biologically diverse location on the planet, one that is rapidly disappearing. I will be able to see the results of how different organisms in such a complex environment adapt to survive, and how various ecological stressors can have a profound effect on evolutionary selection. After conducting this research, I will have hopefully developed a new angle on evolutionary biology and ecology (areas I am hoping to pursue in graduate school), which I will be able to then utilize in my further classroom and laboratory work at Moravian College. Upon completion of this project, an Honors project may also be an option to follow up on the work we will have conducted.

The second reason relates to my future academic plans, both during the summer of 2013 and after my undergraduate work at Moravian College. As this project is projected to take
approximately four weeks to complete, I have been applying to other summer REU (research experience for undergraduates) programs across the country which I would pursue after my SOAR project is completed. I have mainly been trying to locate programs involving ecology or evolutionary biology, my main areas of interest. Participation in the SOAR program would give me prior background in conducting field work, analyzing data and statistics, and reporting my findings in a professionally scientific manner. It would also provide a prior personal research experience that I could share with future REU colleagues and expand upon at other institutions. Although my work at an REU this summer would not be on the exact same topic as my SOAR project, the two would complement each other in basic research area and techniques, developing my skill set as a biologist and preparing me for graduate school. Entry into graduate schools has become increasingly competitive. Today’s applicant must be able to stand out and prove that he or she is able to handle the responsibilities and requirements of a higher education curriculum. One of, if not the most important factor on a graduate school application, is prior research experience. The opportunity to participate in this SOAR project would provide me with the skills and techniques to conduct independent research, giving me a highly valued quality in the eyes of graduate school application committees, and a head start over other entering graduate school students who have not had the chance to conduct research.
SOAR Student Statement of Purpose – Summer, 2013

Project Title: Anti-herbivore defense against leaf-cutter ants in myrmecophyte and non-myrmecophyte Cecropia trees in tropical premontane forests of Manu Park, Perú

Vincent Reed  
Environmental Science  
Class Year: 2014  
Faculty Member: John Bevington  
On-Campus Housing: YES

As an Environmental Science major, it would be foolish of me to pass up the opportunity to travel to Perú and carry out research in one of the most diverse ecosystems on the planet. While I have a great interest in the ecology that surrounds me on a daily basis, the ecology of one of the world’s most diverse ecoregions, the Amazonian Rainforest, is a distinctive environment to study in. By stepping outside the boundaries of the United States, my exposure to a different climate, different culture, and both unique ecological and biological features are a once in a lifetime opportunity. While the experience in itself can and will uncover a part of the world that I never imagined I would visit, it is the research and exploration of the forests of Manu Park that will set this experience apart from any other.

Since I am nearing the end of my junior year, I feel that it is the appropriate time for me to venture further into my field of study and really explore some of the intricacies of our ecological processes. During my stay in Perú, I will also be enrolled in the May Term which is titled “Ecology of Tropical Forests.” By studying this course, my extended stay to carry out research for anti-herbivore defense against leaf-cutter ants will be filled with knowledge of the rainforest and a variety of the plants, insects, and animals that it inhabits. While I have yet to partake in any sort of internship, this SOAR Project will give me my first true field experience that will illustrate many of the topics that I have only read in textbooks or heard through a lecture. By combining my education of environmental science and applying it to tangible research, I am aiming to finally develop my knowledge into something very useful. Stepping outside the confines of a lackluster classroom, walking through the rainforest will provide me first-hand participation in my field of Environmental Science.

What I am most looking forward to is the chance to study such a unique ecosystem. While anti-herbivore defenses exist widely throughout our planet, the opportunity to research and examine such a unique relationship between trees and insects within an even more unique landscape is the chance of a lifetime. I have never traveled outside of our country, let alone anywhere further than Florida. Therefore, being almost 4,000 miles from home I am anticipating the culture-shock that this project will comprise of. Not only that, but I hope the fact that I will be taken out of my comfort zone and exposed to a part of the world that I’ve never been remotely close to will really expand my mind on other parts of the world.

Through this SOAR Project, I expect a variety of things from its outcome. First and foremost, I expect to better understand the ecological relationships that many species have undergone through evolution. The dependence that many species have upon another species is
true indicator of the sophistication that makes up our surrounding environments along with the adaptation for many species’ survival. Secondly, I expect to apply the skills and knowledge that I have gained at Moravian and put them into real life practice. The monotony of lectures, labs, exams, and essays can only implant so much information. Therefore, the first-hand study and field research in a real life tropical forest will go above and beyond anything that books and lectures can teach you. I am also looking forward to gaining a vast new knowledge of topography that I’ve never witnessed before. Thirdly, I expect to learn and understand a variety of research methods, data analysis, and new skills that will better me in my field of study and eventually my career. As I said before, labs and research papers can only prepare you so much so I am hoping that this field experience will push me beyond the usual routine of Moravian semester. Lastly, I am hoping that this SOAR Project can help me open my eyes to the types of careers that lie ahead of me. I never imagined myself going to another country to study and perform research related to academics. Therefore, I hope that this once in a lifetime experience can expose me to certain career possibilities that have never crossed my mind and open my eyes to the opportunities that my field of study presents.