

Organization for Tropical Studies
Undergraduate Semester Abroad Program
Fall 1998

E. I. Deinert, C. T. Ivey, T. E. Shelley, and E. Villalobos, editors

MESSAGE FROM THE DIRECTOR

Fall Semester 1998

by Todd Shelly

The third iteration of the OTS Undergraduate Semester Abroad Program (USAP) came to a highly successful close on December 13, 1998, over much pan de ajo and pizza at the Il Pomodoro restaurant in San Pedro. We had a great group of students this semester – they were bright, enthusiastic, and accomodating, and teaching was a joy. The 25 students in the Program represented 16 colleges and universities, most of which were small private liberal arts colleges (for example, Smith, Bryn Mawr, Swarthmore, Reed were represented). The largest contingent (8 of the 25 students) came from Duke University, which is not unexpected since Duke is the accrediting institution.

The USAP teaching staff for this semester included Drs. Erika Deinert, Christopher Ivey, Todd Shelly, and Ethel Villalobos. Visiting faculty included Drs. Maarten Kappelle of InBio, Deedra McClearn of OTS, William Pfitsch of Hamilton College, and Ethan Temeles of Amherst College. The Centro para Potencial Humanidad (CPH) under the direction of Yamillette Sanabria Rodriguez provided language instruction.

The Program is mobile, and the following chronology includes brief descriptions of our activities in accordance with our itinerary.

September 1 – San Jose

The first morning of the semester was a time of introductions. Students first met one another, and the USAP and OTS staff introduced themselves. USAP staff provided specific information on the itinerary and course syllabi and outlined staff expectations of student academic performance and social behavior. Textbooks were distributed, and other logistic matters were handled.

After lunch, we visited the Instituto Clodomiro Picado of the Universidad de Costa Rica in large part to gain a healthy respect for the venomous snakes of Costa Rica. We heard a lecture on the natural history of the more common species, the biological action of venom, and the treatment of poisonous snake bites. We also saw specimens of several common vipers, including the fer-de-lance (terciopelo) and the eyelash and jumping vipers.

September 2-4 – La Selva

We next traveled to Estacion Biologica La Selva in the Caribbean slope for a brief introduction to a lowland rainforest. Here, the students had an orientation walk through this spectacular forest and heard several lectures on Costa Rican geography and climate. A fruit lab was held, and students much enjoyed their first look at and taste of some exotic and delicious fruits. Here too, students were introduced to the CPH staff, and interviews here conducted to group students according to

their Spanish proficiency. . Last, but hardly least, we made a river rafting trip on the Rio Sarapiquí, which was, needless to say, a huge success.

September 5-25 – Santa Ana

During these 3 weeks, students lived with Costa Rican families and spent 4 hours every weekday morning in Spanish class. The CPH language school is located in the small town of Santa Ana just west of San Jose, and its grassy campus has several small buildings with classrooms, a reading room, and a ranchito among overarching mango trees. Classes were small (5 students, on average), facilitating rapid development of conversational skills, which is the cornerstone of CPH's teaching philosophy. Instruction was rigorous and included daily written assignments, interviews with local people, reading and discussing Costa Rican prose and poetry, and periodic quizzes and exams. A written report on Costa Rican life was required later in the semester.

In addition to more traditional learning, students also participated in cooking typical Costa Rican food and learned traditional and modern dances of Costa Rica. We also made trips to the Museo Nacional, the Museo de Oro, and a local trapiche (sugar cane mill) and heard talks on indigenous peoples, colonial and recent history, folklore and myths, current problems of child labor, and contemporary gender issues. We were also very fortunate to have Deedra Hyde – the preeminent “nature artist” of Costa Rica - speak to our group on the role of art in environmental education.

While emphasis was on language training, we did manage some field trips during this initial portion of the semester. Students were introduced to the spectacular butterfly fauna of Costa Rica in a visit to Finca Mariposas in La Guacima. Two mornings were spent birdwatching, and we saw aracarís, toucans, hummingbirds, woodpeckers, tityras, and mot-mots among other things. We also traveled to Volcan Poas, where students caught a glimpse of the crater and were introduced to high-altitude plants, and Reserva Biológica Carara where we went bird-watching and saw giant crocodiles along the Rio Tarcoles. Carara is adjacent to some Pacific beaches, and after hiking we hit Playa Herradura for some sun and surf.

September 26 – October 17 – Las Cruces

After a big fiesta with students and their host families, we hit the road and headed south to the Estacion Biologica Las Cruces. Once again, Las Cruces proved an excellent site for introducing students to the exuberance of tropical life. Accommodations in the Wilson House are extremely comfortable, the food is great, and the Wilson Botanical Garden is a living laboratory that beautifully showcases the tremendous diversity of tropical plants.

We accomplished several important goals at Las Cruces. First, students received instruction in the identification and natural history of tropical plants. Raul Rojas led an informative tour of the Garden, and students were given lectures and practical exercises in plant vegetative and floral morphology. Two additional lectures – one on ethnobotany and the other on fungi – were delivered by Luis Diego Gomez, station director of Las Cruces. Also, Bill Pfitsch, a botanist from

Hamilton College, visited us for 1 week at Las Cruces and delivered two lectures on plant physiological ecology and led a field exercise that compared photosynthetic rates of sun- vs. shade-dwelling plants. Bill was a hit with the class- he presented complex information in a relaxed, yet orderly, demeanor that the students truly appreciated.

At Las Cruces, students were also introduced to two major agroecosystems in Costa Rica. Field trips were made to coffee fincas and to beneficios to gain first-hand knowledge about the biology and commercial aspects of coffee production. In addition, we visited a nearby oil palm plantation and received an extensive tour of the facility that included much in-depth information on the biology of the palm as well as the economic and social consequences associated with cultivation of this important crop.

On the zoological ledger, we introduced students to tropical insects through collecting and identifying specimens and accompanying lectures on insect biology. A field exercise was also performed that compared insect diversity between two different habitats in the field station. In addition, students learned basic identification and biology of aquatic insects and compared samples at different sites along the Rio Jaba to examine effects of human perturbation on community composition and diversity. Aquatic insects are bizarre creatures, by and large, and the students really enjoyed working with them.

Finally, students were given 4 lectures that introduced hypothesis testing and inferential statistics. Topics included – the normal distribution, independent and paired t-tests and their nonparametric equivalents, ANOVA and the Kruskal-Wallis test, multiple comparison tests, regression, correlation, and contingency tables. Accompanying exercises emphasized problem-solving using JUMP statistical software.

Cerro de la Muerte – October 18-23

In mid-October, we headed north and spent 5 days exploring the beautiful oak forests and paramo found at the Cerro at elevations exceeding 3,000 m. The paramo here represents the northernmost extension of Andean vegetation, and students really enjoyed seeing this exotic habitat. Maarten Kappelle, an InBio researcher who is an expert in high-altitude plants of Costa Rica, led a field project analyzing altitudinal changes in plant communities. Students – especially those from New England – were happy to be cold, happy to wear sweaters and jackets, and happy to stay in a wooden cabin complete with fireplace (and hot chocolate!). Before heading back to San Jose, we also made an early birding trip and got good looks at several resplendent quetzals - the jewel crown of Costa Rica's 850 bird species.

Mid-semester break – October 24 – November 1

This week encompassed our mid-semester break, and students traveled to all parts of Costa Rica - mountains for some, beaches for others. A few, more adventuresome students also traveled to

Managua, Nicaragua, and to Barro Colorado Island, home of the Smithsonian Tropical Research Institute, in the Panama Canal.

Palo Verde – November 2-8

After the week break, we headed northwest of San Jose to Parque Nacional Palo Verde where OTS maintains a field station. Thanks to Hurricane Mitch, we received rain and more rain and then some more rain. Convincing ourselves that wetness is largely a state of mind, however, we plowed forward and accomplished a lot during our stay. One of our major goals at Palo Verde was to allow students more freedom in doing field projects. Previously, we introduced students to field research via faculty-led projects. Here, however, students worked in 2's or 3's on short-term (3-day) projects of their own design. One day prior to departing Palo Verde, students reported their findings in 15-min oral presentations to the entire group. Students did a great job with this, and their projects addressed a variety of interesting topics, including territoriality in jacanas, age-dependent herbivory in ant-Acacia plants, and anti-predator function of wing coloration in butterflies. In addition to the projects, students heard a talk on the biology of mangroves and then made a half-day trip down the Rio Tempisque to see a beautiful mangrove forest. Students had a ball climbing over and through the maze of giant aerial roots of *Rhizophora* that formed a natural jungle gym.

Rincon de la Vieja – November 9-11

Rains from Hurricane Mitch made for impassable roads, and we were unable to travel to Cabo Blanco as originally planned. As an alternative, we visited this beautiful national park in the Guanacaste region. Here, we took a long hike through beautiful the park and saw lots of gigantic strangler figs, toucans, and monkeys as well as fumaroles (sulphur vents) and pailas (bubbling mud pits). Students also had some free time for horseback riding and swimming at a small waterfall.

La Selva – November 12 – December 13

The final month of the semester was spent at Estacion Biologica La Selva. As always, students love their stay here, and who can blame them? Pizotes, sainos, terciopelos, dendrobatid frogs, toucans, oropendolas, sloths, and bullet ants were seen regularly in a setting of lush understory palms and giant 30 m tall canopy-forming trees draped with epiphytes.

At La Selva, we were joined by two visiting faculty. Deedra McClearn, coordinator of OTS's graduate course in tropical ecology, delivered a lecture on neotropical mammals and led a field exercise on secondary seed dispersal. In early December, Ethan Temeles of Amherst College joined us for a week and gave two excellent talks on hummingbird-flower coevolution. He also led a field project that examined the effects of territory size and quality on the foraging and defensive behavior of resident hummingbirds. Both Deedra and Ethan got along very well with the students, and their participation added much to the whole semester. Also, we visited a banana plantation, the Huertos project (a field experiment in tropical forestry), and the ALAS project (a large-scale

arthropod survey). Two researchers at La Selva – Deborah Clark and Reiner Thiele – gave lectures to our group describing their research activities on carbon cycling in tropical forests and the natural history of canopy-dwelling bees, respectively.

The primary focus at La Selva, however, was the completion of long-term independent research projects. Working singly or in groups of 2-3, students were expected to identify a problem, work out the timeline and protocol of data collection, analyze data, and present their findings and interpretation in oral and written reports. USAP faculty took on an advisory role, and students were required to meet with their assigned advisor on a regular basis. The projects were varied and included work on learning in bala ants, territorial behavior of rufous-tailed hummingbirds, the function of leaf drip-tips, extrafloral nectaries and ant defense of *Passiflora* vines, and comparisons between forest edge and interior in the diversity of woody plants and insects. A symposium was held on the final day of formal coursework, and students described their results to their classmates (following the format of a scientific meeting), and the presentations were generally of very high quality.

Over pizza and during farewells, a student told me the best thing about the semester was the gained sense of “connectedness” – with Costa Ricans, the Spanish language, classmates and professors, and, of course, tropical biology. True experiences make powerful memories, and all the students, I suspect, recognized just how much they had seen and learned and how much they had grown as a result.

PARTICIPANTSStudents

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Dr. Ethan Temeles,
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Dr. Maarten Kapelle,
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Dr. Deedra McClearn,
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Guest Lecturers

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Env. Policy Coordinator, OTS

Charley Gomez,
Naturalist Guide

Rafa Campos
Naturalist Guide

Raul Rojas,
Resident Biologist, Las Cruces

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Xinia Miranda,
UNICEF, Children's rights

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COURSE SCHEDULE**SAN JOSE**

August 31 (Mon)

6 pm - Dinner for early arrivals

Sept. 1 (Tues)

8.30 am - Depart hotel for CRO

1.30 pm - Depart CRO for Instituto Clodomiro Picado de la Universidad de Costa Rica for talk/display on venomous snakes (Erika/Ethel; FN)

7 pm - Dinner at Cuartel de la Boca del Monte

LA SELVA

Sept. 2 (Wed)

8 am - Depart hotel for La Selva

1.30 pm - Program Orientation

7.15 pm - Lecture: Global climate (Todd; ES)

Reading: KR - 1 (ES); KR - pp.331-320 (snakes; FN) FM - 1 (ES); JA - snakes: 383, 384, 393, 406 (coral)(FN); JA - butterflies: 730, 741, 751, 775(FN); Rodriguez article(ES); Boza article (ES)

Sept. 3 (Thurs)

7.30-11 am - Field orientation

1.30 pm - Spanish/homestay orientation

7.15 pm - Spanish/homestay orientation con't.

Sept. 4 (Fri)

7.30 - Depart La Selva for river rafting

1.30 pm - Fruit lab (Chris/Ethel; FN)

SANTA ANA

Sept. 5 (Sat)

8 am - Depart La Selva for Centro Para Potencial Humano (CPH)

noon - Lunch at CPH

Sept. 6 (Sun)

8 am Depart CPH for La Guacima Butterfly Farm (Erika/Chris; FN)

Sept 7 (Mon)

7.30-11.30 am - Spanish

1-2 pm - Lecture: Geography and climate of Costa Rica (Chris; ES)

Reading: JA - 3 (ES)

2.15-3.15 pm - Lecture: Indigenous peoples of Costa Rica (Ethel; SP)

Sept 8 (Tues)

7.30-11.30 am - Spanish

1-2 pm - Lecture: History of Costa Rica (Columbus-present, Todd; SP)

2.15-3.15 pm - Lecture: History of conservation in Costa Rica (Jose Maria

Rodriguez; ES)

Sept 9 (Wed)

7.30-11.30 am - Spanish

12 pm (noon) - Group lunch

Sept. 10 (Thurs)

7.30-11 am - Musica y bailes tipicos

1.30-3.30 – Spanish

Reading: Costa Rican Legends (SP); Child labor article (SP)

4-5 pm – Review: Butterflies of Costa Rica (Erika; FN)

Sept. 11 (Fri)

7 am - Depart CPH for Volcan Poas (FN)

3-5 pm Spanish (CPH)

Reading: JA - Poas plants: 225, 236 (Drymys), 239, 308, 311

Sept 12 (Sat)

7.30 am - Depart CPH for Carara (optional)

Sept. 13 (Sun)

Free

Sept. 14 (Mon)

7.30-11.30 am - Spanish

1 pm - Depart CPH for Instituto Nacional de Biodiversidad (InBio; ES)

Reading: Miranda article (ES)

Sept. 15 (Tues)

7.30-11.30 am - Spanish (Desfile)

1-2 pm - Lecture: Contemporary Costa Rica (Erika; ES)

Sept. 16 (Wed)

7.30-9.30 am - Spanish

9.30-11.30 am - Lecture: Role of women in Costa Rica (Thais Aguilar; SP)

12 pm (noon) - Group lunch

Sept. 17 (Thurs)

7.30-11.30 am - Spanish (group A)

5.30 am -noon - Birding (group B; Charley Gomez, SP/FN)

1- 2 pm - Lecture: Birds of Costa Rica (Charley; group B; SP/FN)

Sept. 18 (Fri)

7.30-11.30 am - Spanish (group B)

5.30-noon - Birding (group A, Charley, SP/FN)

1-2 pm - Lecture: Birds of Costa Rica (Charley, group A; SP/FN)

Sept. 19 (Sat)

Free

Sept. 20 (Sun)

Free

Sept. 21 (Mon)

7.30-11.30 am - Spanish

1-2 pm - Lecture: Popular culture/Medicinal Plants (Ethel; SP)

2.15-3.15 pm - Discussion: Meffe/Carroll - Chap. 1,2 (Chris/Erika; ES)

Sept. 22 (Tues)

7.30-11.30 am - Spanish

1-2 pm - Lecture: Legends of Costa Rica (Elias Zeledon; SP)

Sept. 23 (Wed)

7.30-11.30 am - Trapiche

1 pm - Group lunch

Sept. 24 (Thurs)

7.30-11.30 am - Spanish

1-2 pm - Lecture: Art and conservation (DeeDee Hyde, SP)

Sept. 25 (Fri)

7.30-11.30 am - Spanish

12-2 pm – Fiesta

LAS CRUCES

Sept. 26 (Sat)

7:30 am Depart for Las Cruces

7:30 pm - Meeting, equipment inventory and check-in (Classroom)

Sept. 27 (Sun)

8-11 am - Wilson Garden Tour (group A, Raul/Chris; FN)

7-9 am - Garden birding (Erika; FN)

5-6 pm - Lecture: Introduction to Las Cruces (Gail Hewson)

Reading: Chapters on coffee and oil palms

Sept. 28 (Mon)

8-11 am - Wilson Garden Tour (group B, Raul/Erika, FN)

7-9 am - Garden birding (Chris; FN)

1-2 pm - Lecture: Plant morphology (Chris; FN)

2.15-3.15 pm - Lecture: Rainforest structure (Erika; FN)

7.30-8.30 pm - Review: Garden tour (Chris/Erika; FN)

Reading: KR - 2

Sept. 29 (Tues)

6:15 - noon - Oil palm plantation (Erika/Chris; ES)

1:30 - 2:30 pm - Lecture: Species concept and conservation (Todd; ES)

6:00 pm - Barbeque/Mixer

Reading: MC - 3

Sept. 30 (Wed)

7.30-9 am - Rio Jaba walk (group A; Chris; FN)

9-10.30 am - Rio Jaba walk (group B; Chris; FN)

11-12 am - Lecture: Insects I (Erika; FN)

1.30-2.30 - Lecture: Insects II (Erika; FN)

2.45-3.45 - Lecture: Global biodiversity I (Chris; ES)

7:30-8:30 – Review: Garden birds (Erika/Chris; FN)

Reading: MC - 4

Oct 1 (Thurs)

7.30-11 am Coffee finca, beneficio - (group A; Raul/Chris; ES)

7.30-11 am - Insect collection (group B; Erika; FN)

1-2 pm - Lecture: global biodiversity II (Chris; ES)

2.15-3.15 pm - Statistics I: Descriptive stats, normal curve (Todd; FR)

Oct 2 (Fri)

7.30-11 am - Coffee finca, beneficio (group B; Raul/Erika; ES)

7.30-11 am - Insect collection (group A; Chris; FN)

1-2 pm - Lecture: Statistics II: Hypothesis testing, t-test (Chris; FR)

2.15-3.15 pm - Discussion: Coffee/Oil Palms (Chris/Erika; ES)

Oct 3 (Sat)

9 am - Depart Las Cruces for Las Alturas (group A; Chris)

Oct 4 (Sun)

4 pm - Arrive from Las Alturas (group A)

Oct 5 (Mon)

8 - 9:30 am - Flower morphology and biology (Group A; Chris; FN)

- Butterfly techniques (Group B; Erika; FN)

9:30-11 am - Flower morphology and biology (Group B; Chris; FN)

- Butterfly techniques (Group A; Erika; FN)

1 - 2 pm - Introduction to computers and Jump (Group A; Chris; FR)

2 - 3 pm - Introduction to computers and Jump (Group B; Erika; FR)

7.30 pm - Lecture: Physiological ecology of rainforest plants - I (Bill; FN)

Oct 6 (Tues)

8-11 - Inter-habitat insect diversity - collection/sorting/identification

(Erika/Ethel; ES)

1-2 pm - Lecture: Global loss of biodiversity (Chris; ES)

2.15-3.15 pm - Discussion: Biodiversity—MC chap 4, 5 (Erika/Chris; ES)

7 pm - Lecture: Fungi (Luis Diego Gomez; FN)

Reading: MC - 5

Oct 7 (Wed)

8-11 am - Plant physiology field project (Bill; FN)

1-2 pm – Lecture: Statistics III: Correlation and Regression (Erika; FR)

2.15-3.15 pm – Lecture: Insect mating systems (Ethel; FN)

6 pm - Results from Inter-habitat insect diversity project due

7.30 pm - Lecture: Physiological ecology of rainforest plants - II (Bill; FN)

Reading: KR - 3, FM - 2, 3, 4, 5

Oct 8 (Thurs)

8-11 am - Plant physiology field project (Bill; FN)

1-2 pm - Discussion: Results of inter-habitat insect comparison (Erika/Ethel; ES)

2.15-3.15 pm – Lecture: Tropical forest function (Erika; FN)

7.30-8.30 pm - Lecture: Introduction to the paramo (Bill; FN)

Oct 9 (Fri)

8-11 am - Plant physiology field project (Bill; FN)

1-2 pm - Lecture: Speciation in the tropics (Ethel; FN)

Reading: KR - pp. 106-125

Oct 10 (Sat)

9 am - Depart for Las Alturas (group B; Erika)

Oct 11 (Sun)

4 pm - Arrive at Las Cruces (group B)

Oct 12 (Mon)

8-9 am – Introduction to stream insects (Todd; ES)

9- 10:30 – Aquatic insect collecting—Rio Jaba (group A; Todd; ES)

10:30-12 – Aquatic insect collecting—Rio Jaba (group B; Todd; ES)

1:30-2:30 – Lecture: Pollination biology (Chris; FN)

Readings: KR - chap 5-6, FM - chap 6, 7, 8

Oct 13 (Tues)

8-11 am – Rio Jaba/Matadero—collecting, sorting (group A; Todd; ES)

8-11 am – Plant/pollination project (group B; Chris/Erika; FN)

1-2 pm – Lecture: Insect-plant interactions – herbivory (Erika; FN)

7 pm - Lecture: Ethnobotany (Luis Diego Gomez; ES)

Oct 14 (Wed)

8-11 am – Coffee beneficio—insect collecting, sorting (group B; Todd; ES)

9.30-11 am – Plant/pollination project (group A; Chris/Erika; ES)

1-2 pm – Lecture: Statistics IV – Chi-square tests, contingency tables (Todd; FR)

6:00 pm – Finish sorting insects

7:15 pm – Raul's slide show: ecotourism in Costa Rica

Oct 15 (Thurs)

8-8:30 am – Discussion: Aquatic insects (Todd; ES)

8:30-10 am – Discussion: Plant/pollination project (Chris/Erika; FN)

Oct 16 (Fri)

9-10.30 am - ES EXAM

1-2.30 pm - FN EXAM

Oct 17 (Sat)

Free day

Oct 18 (Sun)

9 am - Depart Las Cruces for Cerro de la Muerte

CERRO DE LA MUERTE

Oct 19 (Mon)

8-12 am - Field Project: Gentry transects in primary forest (Maarten; FN)

2-5 pm - Data analysis (Maarten; FN)

7 pm - Lecture: Secondary succession and humans (Maarten, FN)

Oct 20 (Tues)

- 8-12 am - Field project: Gentry transects in secondary forest (Maarten; FN)
 2-4 pm - Data analysis (Maarten; FN)
 4-6 pm - Research symposium (FN)
 7 pm – Lecture: Natural history of the Cerro de la Muerte region (Maarten, FN)
- Oct 21 (Wed)
 1-2 pm – Lecture: Plant mating systems (Chris; FN)
 2:15-3:15 pm – Lecture: Pupal mating in *Heliconius* (Erika; FN)
- Oct 22 (Thurs)
 10 am - Depart for Cabinas Chacón
- Oct 23 (Fri)
 5:30 am - Search for quetzals
 2 pm - Depart for San Jose
- Oct 24 (Sat)- Nov 1 (Sun)
 Vacation

PALO VERDE

- Nov 2 (Mon)
 7 am – Depart La Amistad for Palo Verde
 2-3 pm – Organizational meeting
 3:15-4 pm – Lecture: Introduction to Palo Verde (Eugenio Gonzalez; FN)
 7:15-8:15 pm – Lecture: Sex and the sterile male- tales of the dreaded Gusano Barrenador and Mosca Mediterraneo (Todd; ES)
Readings: MC ch 8, 9, 10, 14(pp 479-495)
- Nov 3 (Tues)
 8-11 am - Field orientation to Palo Verde (FN)
 2-3 pm – Lecture: Natural history of Mangroves (Patricia Delgado/P. Hensel; FN)
 3:15-4:15 – Lecture: Diversidad genetica de *Swietenia humilis* (mahogany, caoba) en Centro America (Maguil; FN)
- Nov 4 (Wed)
 8 am – 2 pm – Mangroves (group A, Patricia; FN)
 All day – Project development (group B)
 Evening: Meet with faculty to discuss projects
- Nov 5 (Thurs)
 8 am - 2 pm – Mangroves (group B, Patricia; FN)
 All day – Project development (group A)
 1:30-2:30 pm – Lecture: Milkweed mating systems and pollination (Chris; FN)
 2:45-3:45 pm – Lecture: Animal defenses: responses to predation (Erika; FN)
 7-8 pm – Lecture: Invasive species: killer bees in the New World (Todd;ES)
- Nov 6 (Fri)
 8 am- 6 pm - Project development (FR)
 1-2 pm – Discussion : primary literature (Chris/Erika; FN)
- Nov 7 (Sat)
 8 am - noon – Project development (FR)

- 1 pm – 6 pm Student presentations (FR)
 Nov 8 (Sun)
 7 am - Depart Palo Verde for Rincón de la Vieja
 Nov 9 (Mon) and Nov 10 (Tues)
 Unscheduled time/Independent project paper write-ups

LA SELVA

- Nov 11 (Wed)
 8 am - Travel to La Selva
 7:30 pm – Lecture: Re-Introduction to La Selva (Francisco Mora; FN)
- Nov 12 (Thurs)
 7 am-noon – Project development (FR)
 1:00 pm - Meeting: Independent projects (FR)
Readings: FM - chap 9, 10, 11; KR - p. 239-244; p. 295-314
 2-6 pm – Project development (FR)
- Nov 13 (Fri)
 8-8:30 am - Introduction to ALAS project (Group A; FN)
 8:30-9 am – Introduction to ALAS project (Group B; FN)
 Rest of day – Project development (FR)
 6 pm - Independent project papers due
- Nov 14 (Sat)
 Unscheduled time/Project development
- Nov 15 (Sun)
 Unscheduled time/Project development
- Nov. 16 (Mon)
 7 am - noon - Project development (FR)
 1-2 pm - Discussion: Primary literature (Chris/ Erika, FN)
 3:15-6 pm - Project development (FR)
 7:30-7:45 pm - Meet with Nora and Amanda (Serena, Jon, Cory)
 7:45-8:00 pm - Meet with Nora and Amanda (Naamal, Margaret, Lisa)
 8:00-8:15 pm - Meet with Nora and Amanda (Brita, Susannah, Andy, Carl)
 8:15-8:30 pm - Meet with Nora and Amanda (Athena, Heather, Kristen)
Readings: MC ch 6, FM ch 12-15, VP ch 1-4, Janzen ch 1
- Nov. 17 (Tues)
 7 am - noon - Project development (FR)
 1- 2 pm - Faculty Project set-up (Nora/Deedra, FN)
 2:15 -3:15 - Lecture: Conservation genetics I (Chris, ES)
 7:30-7:45 pm - Meet with Nora and Amanda (Nicole, Raivo, Greg)
 7:45-8:00 pm - Meet with Nora and Amanda (Brad, Jen, Michelle)
 8:00-8:15 pm - Meet with Nora and Amanda (Jesse, Sarah, Sophia)
 8:15-8:30 pm - Meet with Nora and Amanda (Abbey, Darren, Jessica)
- Nov. 18 (Wed)

- 7 am - noon - Project development (FR)
- 1-5 pm - Faculty Project (Nora/Deedra, FN)
- 7- 10 pm - Independent Project: Meet with Faculty (FR)
- Nov. 19 (Thurs)
 - 7 am - 11 am - Project development (FR)
 - 10:30- 11:30 am - Discussion: Primary Literature (Chris/Erika, FN)
 - 2-5 pm - Faculty Project (Deedra, FN)
 - 7-8 pm - Lecture: Neotropical Mammals (Deedra, FN)
- Nov. 20 (Fri)
 - 7 am - noon - Project development (FR)
 - 11-12 - Lecture: Communication (Erika, FN)
 - 1-2 pm - Discussion: Breakfast of Biodiversity (Chris/Erika, FN)
 - 2-6 pm - Project development (FR)
 - 6 pm - Re-written research papers due in coursebook format
 - 7-8 pm - Lecture: Natural History Exploration in Latin America (Deedra, ES)
- Nov. 21 (Sat)
 - Unscheduled
- Nov. 22 (Sun)
 - Unscheduled
- Nov. 23 (Mon)
 - 7:30-12:00 am - Banana Plantation (Chiquita, ES)
 - 1-2 pm - Lecture: Conservation genetics II (Chris, ES)
 - 4-6 pm - Independent Project: Meet with Faculty (FR)
 - 7-8 pm - Lecture: Carbono Project (Debra, ES)
 - 8-9 pm - Independent Project: Meet with Faculty (FR)
- Nov. 24 (Tues)
 - 7:30-9:30 am - Huertos Project, Group A (Miguel, ES)
 - 9:30-11:30 am - Huertos Project, Group B (Miguel, ES)
 - 1-2 pm - Lecture: Frogs of La Selva (Erika, FN)
 - 2-6 pm - Project Development (FR)
- Nov. 25 (Wed)
 - 8 - 10 am - FN EXAM II
 - 1:30 - 3:30 pm - ES EXAM II
- Nov. 26 (Thurs)
 - Thanksgiving
- Nov. 27 (Fri) – Nov. 29 (Sun)
 - unscheduled
- Nov. 30 (Mon)
 - 7 am-dark - Project Development (FR)
 - 6 pm - Spanish Paper due
 - 7-8 pm – Lecture: Pollination Biology (Ethan, FN)
- Dec. 1 (Tues)
 - 7 am-noon - Project Development (FR)

- 1-4 pm – Independent Project: meet with faculty (FR)
- 6 pm - Palo Verde Rewrites due
- 7-8 pm – Lecture: Hummingbird-Flower Interactions (Ethan, FN)
- 8:15 – 9:30 pm - Independent Project: meet with faculty (FR)
- Dec. 2 (Wed)
 - 7am-noon – Faculty Field Project (Ethan, FN)
 - 7-8 pm – Lecture: Canopy Bees (Reiner, FN)
- Dec. 3 (Thurs)
 - 7am-noon – Faculty Field Project (Ethan, FN)
 - 1-2 pm – Discussion: Primary literature (Todd/Erika; ES)
 - 7-8 pm – Lecture: Genetic consequences of habitat fragmentation (Chris, ES)
- Dec. 4 (Fri)
 - 7-noon – Project Development (FR)
 - 1-3 pm – Faculty Field Project (Ethan, FN)
 - 7-8 pm – Lecture: Ants (Erika, FN)
 - Independent Project: Begin writing
- Dec. 5 (Sat)
 - Unscheduled
- Dec. 6 (Sun)
 - Unscheduled
- Dec. 7 (Mon)
 - 1:30-2:30 Trials Project (Pablo; ES)
 - 6 pm - Independent Project Paper Due in Coursebook Format
- Dec. 8 (Tues)
 - 1-2 pm - Discussion: Breakfast of Biodiversity ch. 5-10 (Chris/Erika; ES)
- Dec. 9 (Wed)
 - 8-9:30am - ES Final Exam
 - 10-11:30 am - FN Final Exam
- Dec. 10 (Thurs)
 - Work on Rewrites and Presentations
- Dec. 11 (Fri)
 - 9-noon – Student Presentations
 - 1-3 pm - Student Presentations
 - 3 pm – Pack up all boxes
 - 6 pm - Final Papers Due in Coursebook Format
- Dec. 12 (Sat)
 - 6:30 am-9 pm - to the beach
- Dec. 13 (Sun)
 - 7 am – Begin loading the Toyotona and the bus
 - 8 am - Depart for San Jose
 - 6 pm – Final Dinner at Il Pomodoro
- Dec. 14 (Mon)
 - Departures begin

RESEARCH PROJECTS

LAS CRUCES

Physiological and morphological acclimation to light availability in a light-loving plant, *Pouteria caimito* (Sapotaceae)

Category: Faculty Field Project

Participants: Athena Dodd; Nicole Donovan and Brad Feldman (contributors); Bill Pfitsch (resource person)

Site: Las Cruces

Key words: Acclimation, photosynthesis, *Pouteria caimito*, shade-intolerance

Introduction

The relationship between phenotypic plasticity and the ecological distribution of a species is a central question in plant ecology. As sessile organisms, plants are committed to the location in which they germinate and must be able to withstand varying conditions in order to survive and successfully reproduce. Because light availability is a factor that determines plant survival and success in tropical forests, the distribution of many plant species can be explained in reference to this.

Earlier studies have found differences in photosynthetic acclimation between shade-tolerant and shade-intolerant species, where light-loving species were more able to acclimate to changing light availability than their shade-tolerant congeners (Chazdon 1992; Chazdon and Kaufmann 1993). In these studies little attention was paid to the range in acclimation responses that might be exhibited within a species—an important factor in gauging potential ecological distribution in space or time. The objective of this study was to assess the acclimation ability of a light-loving shrub, *Pouteria caimito* (Sapotaceae) within and between individual plants. Both physiological and morphological measurements were used to describe photosynthetic capacity, including maximum rate of CO₂ assimilation, stomatal

density, and leaf mass per area. It was predicted that *P. caimito*, as a light-loving plant, would display acclimation to different levels of light availability, and that this response would vary between plants.

Methods

This study was conducted on October 8, 1998 at Las Cruces Biological Station in the Wilson Botanical Garden, Costa Rica. The subject plant, *Pouteria caimito* (Sapotaceae) is a woody shrub naturally occurring in mid-elevation, wet-montane forests of South America. Two adjacent plants were selected on the basis of their location in an open-light environment and the availability of leaves in both high- and low-light locations on the plant.

A camera light meter was used to characterize areas of high and low light availability on the plants at noon on an overcast day. With a constant aperture of f16, low-light locations required shutter speeds of between 1/8 and 1/30 seconds to deliver the same amount of light as 1/250 to 1/1000 seconds in the high-light treatment. Fourteen leaves were collected from each plant, seven from each light level. Leaf-age was regulated by choosing leaves one whorl proximal to the newest leaves on a branch. Leaves were kept in water until photosynthetic rates had been measured,

and then stored in a sealed plastic bag before taking fresh mass measurements.

The rate of CO₂ assimilation, A_{\max} , was measured using a portable photosynthesis system (Li-Cor model 6200) with a light source of 800-1000 $\mu\text{mol photons}/\text{cm}^2/\text{sec}$. Measurements were taken in a laboratory setting with a starting CO₂ concentration of 300 PPM. Due to time constraints these data were only taken for plant A. Stomatal densities were calculated as an average of three stomatal counts in three different fields of view (400x magnification) on epidermal peels taken from the underside of each leaf. Leaf area was measured using a leaf area meter (Li-Cor 3100) and fresh leaf mass was measured on an analytical balance. Leaf mass per area (LMA), a measure of leaf thickness, was calculated as the ratio of leaf mass to leaf area.

Statistical analyses were conducted with the statistical software package JMP 3.2.2 (SAS Institute, Inc.). For plant A, A_{\max} was compared between the light categories using Student's T-test. A two-factor analysis of variance with an interaction effect was used to compare leaf mass, stomatal density, and LMA between plants and light treatments. Pearson's correlations were calculated between A_{\max} , stomatal density, and LMA.

Results

Leaf characteristics differed more between light levels than between plants (Tables 1 and 2). On average, leaves that grew in the light had 50% more leaf mass per area and 53% more stomata than leaves from shaded areas (Table 1). Leaves from well-lit locations showed a significantly faster rate of CO₂ assimilation: about 183% faster than shaded leaves ($t =$

-5.194 , $df = 7$, $P = 0.0013$; Table 1). Plants differed significantly only in LMA, where plant B had leaves that were 14% thicker on average. A weak interaction trend was also observed in how LMA changed in each plant between different light levels (Table 2). Plant A increased the thickness of its leaves by 53% between dark and light treatments, as opposed to 25% in plant B. Plant B however, had a 28% higher LMA measurements under low-light growth conditions than plant A and maintained higher LMA values overall.

Correlation analyses revealed that A_{\max} was more strongly correlated with leaf mass per area ($r = 0.8630$, $P = 0.0027$) than with stomatal density ($r = 0.6654$, $P = .0505$).

Discussion

Intra-plant differences in morphological and physiological characteristics of *P. caimito* were detected in leaves grown under two light levels. Leaves grown in high-light conditions were typically thicker, had higher stomatal density, and photosynthesized at a considerably higher rate than those grown under low-light conditions. Stomatal density is a morphological indicator of a plant's photosynthetic potential because of its role in determining the amount of gas exchange that occurs for photosynthesis. Leaf thickness is also a useful indicator of increased photosynthetic potential because it reflects cellular changes in the amount of photosynthetic tissue per unit leaf area (Sims and Pearcy 1992). The correlation of LMA and stomatal density with A_{\max} shows that this relationship between morphological characteristics and physiological capacity holds for *P. caimito*. Therefore elevated morphological

investment in light-treated leaves should reflect increased investment in photosynthetic machinery on a physiological level. *Pouteria caimito* can acclimate photosynthetically to benefit from prevailing light conditions on a leaf-by-leaf basis.

Leaves grown under low-light conditions were significantly thinner and smaller in terms of fresh mass. Chazdon (1992) describes this shift in resource allocation as a down-regulation of physiological processes in shaded leaves. Such a system of down-regulation would result in a smaller investment by the plant in a leaf that is in a less advantageous position for performing photosynthesis. By investing biomass where photosynthesis has the opportunity to occur at a higher rate, *P. caimito* can increase its overall photosynthetic efficiency and may compete more effectively for space in areas of patchy light availability.

Leaf thickness increased twice as much in plant A as in plant B between low- and high-light categories although plant B maintained higher LMA levels overall. This suggests that plants have different capabilities depending on the light level in which leaves are grown. Under natural conditions these differing capabilities may translate into varying success related to the prevailing microclimate, especially when taking into account the possibility that the individuals used in this study were not genetically distinct and the results represent an under-estimate of existing intra-specific variation. If the correlation between LMA and photosynthetic rate is as strong as the data suggest, intra-specific variation in this

characteristic could indicate wider ecological distribution of the species than predicted by indiscriminate sampling of leaves from several plants. Larger sample sizes of leaves and plants would be required to verify the statistical significance of this finding, and ecological implications for the distribution of the species would only be expected if such intra-species variation was genetically-based.

Literature Cited

- Chazdon, R.L. 1992. Photosynthetic plasticity of two rain forest shrubs across natural gap transects. *Oecologia* 92:586-595.
- Chazdon, R.L. and S. Kaufmann. 1993. Plasticity of leaf anatomy of two rain forest shrubs in relation to photosynthetic light acclimation. *Functional Ecology* 7:385-394.
- Sims, D.A. and R.W. Pearcy. 1992. Response of leaf anatomy and photosynthetic capacity in *Alocasia macrorrhiza* (Araceae) to a transfer from low to high light. *American Journal of Botany* 79:449-455. *cited in* Chazdon, R.L. and S. Kaufmann. 1993. Plasticity of leaf anatomy of two rain forest shrubs in relation to photosynthetic light acclimation. *Functional Ecology* 7:385-394.

Table 1. Means and standard deviations of leaf measurements from *Pouteria caimito* grown under two different light levels. $n = 7$ for each category, except where indicated in parentheses.

	Low-Light		High-Light	
	Plant A	Plant B	Plant A	Plant B
A_{\max} ($\mu\text{mol}/\text{m}^2/\text{sec}$)	3.626 \pm 2.313 (4)		10.214 \pm 1.500 (5)	
Leaf Mass (g)	1.17 \pm 0.34	1.10 \pm 0.37	1.83 \pm 0.44	1.75 \pm 0.33
Stomata /field of view, 400x	16.1 \pm 3.9	19.6 \pm 3.7	24.7 \pm 3.0	25.9 \pm 4.6
Leaf Mass/Area (g/m^2)	175.5 \pm 15.7	224.4 \pm 32.4	268.1 \pm 27.6	281.6 \pm 25.2

Table 2. Summary statistics of two-factor ANOVA comparisons of leaf mass, leaf mass area, and stomatal density, including interaction effects between light levels and plant individuals of *Pouteria caimito*.

Effect	df	SS	F	P
Leaf Mass (g)				
Plant	1	0.0364	0.2631	0.6127
Light level	1	3.0294	21.878	<.0001
Plant*Light level	1	0.000004	0.0000	0.9960
Error	24	3.3232		
Stomata/field of view, 400x				
Plant	1	36.571	2.516	0.1258
Light level	1	386.286	26.575	<.0001
Plant*Light level	1	9.143	0.629	0.4355
Error	24	348.857		
Leaf Mass/Area (g/m^2)				
Plant	1	6821.63	10.138	0.0040
Light level	1	39275.14	58.369	<.0001
Plant*Light level	1	2188.93	3.253	0.0839
Error	24	16149.03		

Photosynthetic plasticity of *Pouteria caimito*

Category: Faculty Field Project
Participants: Nicole Donovan, Athena Dodd, and Brad Feldman
Site: Las Cruces
Keywords: light environment, photosynthetic rate, plasticity, *Pouteria caimito*

Introduction

Varying levels of light in the rain forest have led to the development of photosynthetic and morphological plant adaptations. While shade-tolerant species experience lower, more constant levels of light in the rain forest understory, shade-intolerant plants must develop adaptations to daily variations in sunlight. In response to this greater fluctuation of light, shade-intolerant species are expected to have greater photosynthetic plasticity than shade-tolerant species (Bill Pfitz, pers. com.). Adaptations to constant changes in light include increased capabilities of altering light-harvest machinery, electron capacity and carbon capacity (Chazdon 1992). Light-level exposure for leaves of certain shade-intolerant species, however, varies within the same tree. Leaves located closer to the center of the tree occupy a much darker habitat than those leaves on the outer branches. I investigated the plasticity of maximum photosynthetic rates, leaf mass area, and stomatal density between leaves occupying sun and shade environments from a single, shade-intolerant *Pouteria caimito* individual. I hypothesized that leaves from the sun environment would yield higher levels of photosynthesis, as well as display distinct morphological advantages to aid in the increased photosynthetic demand.

Methods

A member of the family Sapotaceae, *P. caimito* is found throughout Central America and northwestern South America, favoring lowland and lower montane rain forests. To investigate differences between *P. caimito* leaves found in sun and shade environments, I located an individual tree in an open area of the Wilson Botanical Gardens at Las Cruces, Costa Rica. The tree was located on the east side of the Gardens, near the bamboo stands. The species was chosen based upon its shade-intolerant characteristics, as well as its self-shading abilities. I determined a light and dark environment with the use of a camera light meter. With an aperture of F16, the sun environment had a shutter speed of 1/250 to 1/1000 of a second, while the shade environment had a shutter speed of 1/8 to 1/30 of a second. Shutter speed readings were recorded on October 7, 1998, during an overcast afternoon. The following morning, I collected 7 leaves from each of the environments, taking only the youngest, fully emerged leaves. After carefully labeling each leaf, I placed individual leaves in a small container of water and exposed them to direct sunlight for thirty minutes. Leaves were then tested for maximum photosynthetic rate using the Li-Cor 6200 Photosynthesis System. Leaf mass was determined by weighing individual leaves with an electronic balance. A Li-Cor 3100 leaf area meter was used to find leaf area of

each sample. Leaf mass area was calculated by dividing mass (g) by area (cm^2). Leaves were also tested for stomatal density. Nail polish was applied to an area approximately 1 cm^2 , located on the underside of the leaf. After allowing the polish to dry, the polish was removed and used to prepare a wet-mount slide. Three different areas were counted from each leaf, under a 40x lens. An average of the three counted areas was used to obtain final stomatal density numbers. T-tests were used to compare differences between sun and shade treatments. Relative differences between mean sun and shade leaves were calculated by dividing the difference of mean sun and shade values by the mean shade value. Correlations were also made to compare trends in leaf morphology (leaf mass area and stomatal density) with maximum photosynthetic rate.

Results

Maximum photosynthetic rate, leaf mass area, and stomatal density were all significantly higher in sun leaves than in shade leaves (Table 1). However, maximum photosynthetic rate had a greater relative difference than leaf mass area and stomatal density (Table 1).

Maximum photosynthetic rate was positively correlated with leaf mass area ($r = 0.863$, $P = 0.0027$; Figure 1). A positive correlation between maximum photosynthetic rate and stomatal density was also made ($r = 0.6654$, $P = 0.0505$; Figure 2). The correlation between maximum photosynthetic rate and leaf mass area is slightly stronger than the correlation between maximum photosynthetic rate and stomatal density.

Discussion

Collected data support my hypothesis that *P. caimito* leaves from a sun environment display greater rates of photosynthesis than those of a shadier environment. My findings are in accordance with Chazdon, who has observed greater photosynthetic plasticity in shade-intolerant species (1992). Other tests have also shown that shade species tend to have lower maximum photosynthetic values (Luttge 1997). Thus, leaves collected from the shade environment may have adapted greater shade-tolerant attributes to adjust to the low-light environment. This acclimation to a shade environment includes a down-regulation of biochemical processes, thus supporting the observed lower maximum photosynthetic rates seen in the shade leaves (Chazdon 1992).

Data also support my hypothesis that leaves from the sun and shade environments have distinct morphological differences. Both leaf mass area and stomatal density have mean sun values approximately 50% > the respective mean shade values. Leaf mass area has repeatedly been reported to be greater in sun leaves, as my data also suggest (Luttge 1997). Differences in leaf mass area may result from a sun leaf's need for more light harvesting machinery. Thus, sun leaves would be better equipped to utilize the large amounts of solar energy to which they are exposed.

Strong positive correlations between maximum photosynthetic rate and both leaf mass area and stomatal density support my hypothesis that sun leaves display distinct morphological differences to aid in the increased photosynthetic demand. Sun-loving leaves have been found to have greater amounts of chlorophyll, leading to

increased maximum photosynthetic rate (Chazdon 1992). Similarly, an increase in stomatal density also results in higher photosynthetic rates, as the plant has a greater capacity to capture carbon dioxide.

Future studies should attempt to obtain light data for each leaf collected. With light measurements for each leaf, continuous, rather than discrete, relationships can be examined between physiological and biochemical differences with respect to light. A greater sample size of shade and sun leaves should also be collected, as a small sampling can lead to a greater chance of inaccurate results. More individuals of *P. caimito* should also be tested to observe variation in shade-tolerant adaptations of inner leaves among individuals of this species of tree. Despite the need for future testing, collected data strongly support the hypothesis that leaves from the sun environment yield higher levels of photosynthesis, as well as display distinct morphological advantages to aid in this increased photosynthetic demand. Such

relationships between high and low light environments with respect to plant physiological responses prove to be useful knowledge in further comprehension of forest growth and production.

Literature Cited

- Chazdon, Robin L. 1992. Photosynthetic plasticity of two rain forest shrubs across natural gap transects. *Oecologia* 92: 586-595.
- Gentry, Alwyn H. 1993. A field guide to the families and genera of woody plants of northwest South America. University of Chicago Press, Chicago, Illinois, USA.
- Heywood, V. H. 1993. Flowering plants of the world. Oxford University Press, New York, USA.
- Luttge, U. 1997. Physiological ecology of tropical plants. Springer, New York, USA.

Table 1. Summary of differences in maximum photosynthetic rate (Max Ps), leaf mass area (LMA), and stomatal density for shade and sun leaves of *Pouteria caimito* at Wilson Botanical Gardens, Las Cruces, Costa Rica. **= $P < 0.01$, ***= $P < 0.001$, ****= $P < 0.0001$ in a *t*-test comparing means of sun and shade leaves.

Test	n	Mean Shade (SD)	Mean Sun (SD)	Relative Difference
Max Ps Rate ($\mu\text{mol}/\text{m}^2/\text{sec}$)**	7	3.63 (\pm 2.313)	10.21 (\pm 1.8)	1.83
LMA (g/cm^2)***	7	0.018 (\pm 0.34)	0.027 (\pm 0.44)	0.50
Stomatal Density (per 40x view)****	7	17.86 (\pm 3.9)	25.29 (\pm 3.0)	0.53

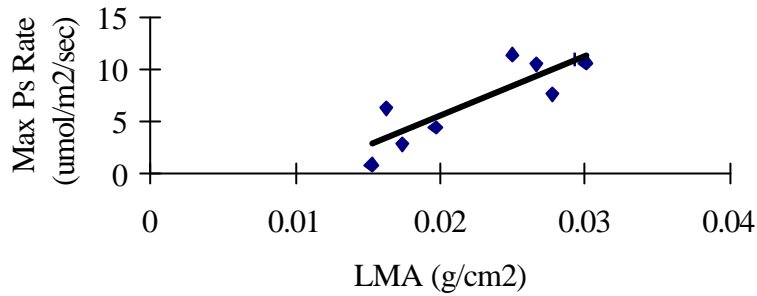


Figure 1. Maximum photosynthetic rate vs. leaf mass area of *P. caimito* at Wilson Botanical Gardens, Las Cruces, Costa Rica.

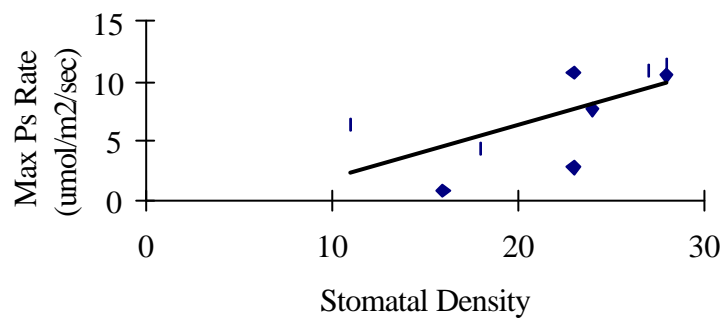


Figure 2. Maximum photosynthetic rate vs. stomatal density of *P. caimito* at Wilson Botanical Gardens, Las Cruces, Costa Rica.

Felony and the flowering plant: effects of nectar robbery on *Stachytarpheta jamaicensis* pollen loads

Category : Faculty Field Project

Participants : Darren Miao and Athena Dodd

Site : Las Cruces

Key Words : Nectar robbery, pollen load, pollination, *Stachytarpheta jamaicensis*

Introduction

Due to their sessile nature, flowering plants must rely on insects and birds for pollen transfer between individuals (Inoye 1981). To attract pollinators, many flowers produce nectar rich in sugar and other carbohydrates to entice species into visiting, as visitation is often equated with pollination (Inoye 1981). However, cheaters exist within this system. Certain species have learned to obtain nectar by biting through the corolla at the base and thus bypassing the flower's sexual organs. This activity, referred to as "nectar robbing" would seemingly be detrimental to plant reproduction, as robbing species do not aid in pollen dispersal. Additionally, robbery would result in lower concentrations of nectar within affected plants, possibly deterring potential pollinators from visiting, and pollinating, these less rewarding plants.

The purpose of this study is to investigate the effects of nectar robbery on floral pollination, specifically within the species *Stachytarpheta jamaicensis*. Since pollen of *S. jamaicensis* is deposited on floral stigmas when pollinators visit flowers to drink nectar, stigmal pollen load can be used as an indicator for effective pollination. It is hypothesized that robbed flowers would have lower pollen counts than un-robbed flowers.

Methods

Stachytarpheta jamaicensis grows naturally in Costa Rica along Atlantic beaches, the Pacific slope at low to middle elevations, and in the Guanacaste region (Standley 1938). It also grows as an introduced species in the Wilson Botanical Garden (San Vito, Costa Rica) where the study was conducted. The small, purple flowers of this species bloom in small bunches on stalks called racemes. Pollen grains resemble small fish eggs under the dissecting scope (pers. obv).

For the study, ten racemes were randomly collected. Each possessed 4 to 7 blooming flowers, for a total of 64 flowers. In the laboratory, flowers were separated into two groups : corollas that exhibited signs of nectar robbery and those that did not, with the frequency of robbery then calculated. Holes or slits located at the base of the corolla were taken as signs of nectar robbery. Once the separation was complete, twenty specimens from each group were randomly selected and viewed under dissecting scopes. Counts of the number of pollen grains upon individual stigmas were made.

The amount of pollen on the stigmas of robbed and unrobbed plants was compared using a Wilcoxin Rank Sum test and the JMP statistical software package.

A quick survey of the types of species that were present around *S.*

jamaicensis was also taken at the end of the study.

Results

Of the 64 flowers collected, 24 (37.5%) exhibited signs of nectar robbery. The number of pollen grains present on robbed flowers and un-robbed was not normally distributed. Most robbed flowers contained no pollen. Four stigmas (20%) possessed a grain of pollen.. Pollen counts for un-robbed flowers ranged from 0 - 5, with 10 possessing no pollen (50%), 5 possessing a single grain (25%), 1 possessing two grains (5%), 2 possessing four grains (10%), and 2 possessing five grains (10%). Un-robbed flowers thus had a significantly higher pollen count than robbed flowers ($Z = 2.22461$, $df = 38$, $P = .0261$). Butterfly scales were observed in abundance on all flower stigmas, regardless of pollen count.

Observed species that visited *S. jamaicensis* flowers included the rufus tailed hummingbird, the *Trigona* stingless bee, 5 types of Lepidoptera (4 butterflies and one unidentified species), and 2 unidentified species of Hymenoptera (one ant, one wasp).

Discussion

As hypothesized, stigmas of robbed flowers contained less pollen counts than un-robbed flowers, indicating that nectar robbery results in reduced pollination of *Stachytarpheta jamaicensis*. This finding has several implications for *S. jamaicensis* reproductive success. Primarily, a reduced pollination rate would decrease an individual's outcrossing. This decrease is fairly important if outcrossed individuals produce more viable offspring. Mutations are less likely to occur in

outcrossed individuals and genetic defects from one parent can be negated by the genetics of the other parent. Thus, it is evolutionary beneficial for individuals to outcross. Therefore, nectar robbery would have a negative effect on *S. jamaicensis* fitness. Additionally, since plants often require more than one pollen grain for fertilization, nectar robbery would further hurt *S. jamaicensis* because less pollen grains would be present upon the stigmas of robbed individuals than on the stigmas of un-robbed individuals. This decrease in pollen grain load would most likely translate into lower fertilization rates and decreased number of offspring. However, further studies investigating the fertilization rates of robbed and un-robbed flowers need to be conducted in order to verify this hypothesis.

Several possible explanations can be given for the differences in pollen grain load. First, legitimate pollinators may be able to distinguish between robbed and un-robbed flowers, though the mechanism for this knowledge remains unknown. Since robbed flowers would most likely contain significantly lower levels of nectar, pollinators may forgo visiting such flowers, thus resulting in lower pollen counts in such individuals. However, further studies measuring the nectar levels of robbed and un-robbed flowers need to be conducted before such a conclusion can be drawn.

Another possible explanation for the lower pollen counts in robbed flowers is that damage to the nectaries may trigger an induced floral response where the stigma of the flower loses its stickiness. Such a state would prevent pollen from sticking to the stigma, thus resulting in the lowered counts. However, the large number of butterfly scales attached to both types of stigmas suggests that this is not the case here. If

robbed flowers did exhibit this induced response, then a significantly reduced number of butterfly scales should have been observed in robbed flowers.

Finally, it is possible that some of the legitimate pollinators may also be nectar robbers. If a legitimate pollinator decides to nectar rob instead of obtaining nectar the conventional way, then pollen loads would decrease as the number of legitimate visits would also decrease. Again, further studies observing the foraging behaviors of legitimate pollinators of *S. jamaicensis* is needed to support this hypothesis.

Although nectar robbing was not directly observed in this study, several possible culprits can be identified based on previous studies. *Trigona* bees (Brandenburg 1976), ants (Free 1970), and wasps (Rust 1977) have all been observed robbing nectar in various flowers, although never in *S. jamaicensis*. Lepidoptera could also be engaging in nectar robbing, as their proboscis may be small enough to bypass the relatively large pollen of *S. jamaicensis*. However, actual observations need to be made in all cases before a species' role as *Stachytarpheta jamaicensis* nectar robbers can be determined.

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The effects of nectar robbery on stigma pollen load of *Thunbergia grandiflora*

Category: Faculty Field Project
Participants: Raivo Vihman and Heather Fowler
Site: Las Cruces
Key Words: Nectar robbery, reproductive success, *Thunbergia grandiflora*, *Trigona*

Introduction

A common strategy of plants for attracting pollinators to their flowers is to provide a source of nectar at the base of the corolla. In the process of feeding on the nectar, pollinators brush the anthers and stigma with part of their body, depositing pollen from other flowers while picking up new pollen. Many flowers have evolved forms specifically suited to effective pollinators, such as the long corolla tubes associated with many butterflies and hummingbirds. Some insects that would be excluded by flower morphology have evolved alternative strategies of “stealing” nectar while bypassing the flower’s sexual parts; these are known as nectary robbers. Nectar robbery usually involves chewing a hole through the base of the corolla to gain access to nectar without entering through the corolla tube (Inouye 1981).

Nectar robbery is a foraging method commonly employed by short-tongued bees such as some *Bombus*, *Xylocopa*, and *Trigona* species, as well as other insects and some short-beaked hummingbirds (Barrows 1980). Primary nectar robbery is generally considered to increase foraging efficiency in relation to legitimate flower entry, and in some cases may be the only access to nectar for robbers (Inouye 1981). The effects of nectar robbery on seed set, or reproductive success of the plant has been controversial.

Nectar robbers are expected to have a negative impact on the plant’s reproductive success, but research in this area has been inconclusive. Various studies have shown decreased seed set, no effect, as well as increased seed set in nectar robbed flowers (Inouye 1981). Robbers may, for example, indirectly pollinate self-fertile flowers through their movements on the flower and may even cross-pollinate flowers by contacting sexual flower parts when visiting flowers (Alford 1975). This study aims to determine whether nectar robbery has a negative effect on reproductive success of *Thunbergia grandiflora*, measured through stigma pollen load.

Methods

This study was conducted on October 14, 1998 at the Las Cruces Biological Station in the mountains of southern Costa Rica. Sixty flowers were haphazardly selected from a plot of *T. grandiflora* vines and checked for holes at the bases of their corollas for evidence of nectar robbery. Twenty robbed and twenty unrobbed flowers of similar age were collected. Pollen grains were counted on each stigma under a dissecting microscope. Data were analyzed with a *t*-test using the JMP statistical package (Sall and Lehman 1996).

Results

Out of 60 flowers sampled, 40 (67%) had been robbed. Eight of the 20 unrobbed flowers had holes chewed most, but not all the way through the base of the corolla. *Trigona* stingless bees were the most commonly observed robbers, but were observed entering and exiting flowers legitimately as well. At least two species of wasps were observed between the calyx and corolla, suggesting robbery on their part as well. At least three species of ants were observed on the flowers, often at the same time as the *Trigonas* or wasps.

The pollen load ranged from 0 to 14 pollen grains per stigma for both robbed and unrobbed flowers. The mean pollen loads for robbed and unrobbed flowers were 4.15 (SD = 4.146) and 3.45 (SD = 3.268), respectively. A *t*-test between robbed and unrobbed revealed no significant difference in stigma pollen load ($t = 0.593$, $df = 38$, $P = 0.5567$).

Discussion

Thunbergia grandiflora has been cited by Faegri and van der Pijl (1966) as an example of a plant adapted against nectar robbing through a mutualistic relationship with ant guards. These guards are reported to repel nectar robbers such as *Xylocopa* bees from the base of the corolla, and plants that do not engage in this mutualism are said to be rare. Our observations contradict this, in that most of the flowers we observed contained no ants, and where ants were present, nectar robbers were present as well. It is possible that the ant species involved in the mutualism with *T. grandiflora* is not present in the garden at Las Cruces, because *T. grandiflora* is native to Asia and therefore an introduced species to the Americas. Inouye (1981) suggests that *T.*

grandiflora also has a thickened corolla which is thought to be protection against nectar robbery. Clearly, if the thick corolla is an adaptation against nectar robbing it is only capable of slowing down the robbers, and not of eliminating them. Possibly in conjunction with the ant guards, which do not seem to be present in the study population, the thickened corolla could increase the effectiveness of the ant protection by providing them with more time to attack potential robbers, thereby decreasing successful nectar robbery attempts. The eight unfinished holes support this contention.

Free and Williams (1973) found a positive correlation between frequency of robbery and corolla tube depth and width. It seems counterintuitive that wide corolla tubes should be positively correlated with nectar robbery, but this relationship is supported by our observations of *T. grandiflora* flowers, which are relatively deep tubes, and much wider than the bodies of any of the nectar robbers we observed. The economy of robbing *T. grandiflora* makes more sense when it is noted that the corollas, although wide at the mouth, have a constriction above the ovaries which may not allow legitimate access of short-tongued bees and wasps (and ants, for that matter) to the nectar. Another advantage of nectar robbery may be that it allows robbers access to the nectar before the flower has opened, whereupon pollinators would have legitimate access. This behavior was observed later by a *Trigona* bee in the same population. This could benefit the efficiency of robbing, by ensuring an unexploited nectar source waiting at the other side of the thick corolla. Despite the thickened corolla, 67% of flowers sampled

were robbed, and including the 8 uncompleted robberies, 80% were robbed or in the process of being robbed. This suggests that robbing *T. grandiflora* is an efficient foraging technique at Las Cruces.

Interpretation of our results is complicated, as our study population was not in its native ecological context. An essential symbiotic species of ant guard may be missing, as well as its specific pollinators. Therefore inferences of these data upon *T. grandiflora*'s evolutionary history in relation to nectar robbing are difficult. Although nectar robbing appeared to be common in *T. grandiflora*, statistical analysis suggests that it had no effect on stigma pollen load in our study population. It is difficult to interpret these data meaningfully in relation to reproductive success, however. It is not known, for example, whether a pollen load of 14 grains per stigma (the maximum load observed) is a high or low pollen load in natural populations of *T. grandiflora*, and therefore how such a pollen load would translate to seed set. It is possible that *T. grandiflora* lacks an effective pollinator at Las Cruces, and that the pollen load observed was in fact very low; the surfaces of the stigmas seen under the microscope were almost completely barren, even under our highest pollen count. Greater knowledge of *T. grandiflora*'s reproductive biology is necessary for meaningful analysis of our results.

In the absence of its native pollinator(s), it is possible that the observed pollen load was due solely to the nectar robbers. If this is true, and if the observed pollen load translates to seed set, this would lead to the conclusion that in this environment, nectar robbers can benefit *T. grandiflora* reproductive success. Studies

relating pollen load to seed set, seed set to nectar robbery, as well as *in situ* natural history observation at the Las Cruces site and in *T. grandiflora*'s native habitat, are necessary to reveal the effects of nectar robbing on reproductive success within and outside its native context.

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CERRO DE LA MUERTE

Primary and secondary cloud forest structure on Cerro de la Muerte

Category: Faculty Field Project
Participants: Serena Black, Brad Feldman, Kristen Ford, Sophia Kuo, Carl Salk and Lisa Stano
Site: Cerro de la Muerte
Key Words: Cloud forests, forest structure, oak forests, succession

Introduction

The contrasting ecological histories of primary and secondary forests are evidenced by differences in forest structure and tree species composition. For example, high altitude tropical primary forests are often characterized by a few dominant tree species that have grown to maturity, outcompeting early successional species in the process. High altitude secondary forests, in contrast, generally have a higher species diversity and a greater density of young, fast-growing trees; these colonizing species are eventually replaced by slower-growing, longer lived trees (Maarten Kapelle, pers. comm.). In addition, the species composition of a primary forest tends to be stable, whereas secondary forests are in a state of flux with high species turnover rates. Thus we can expect primary and secondary forests to differ significantly in type, abundance and distribution of species, as well as in tree size.

In this study, we compared a transect of primary cloud forest with a transect of secondary cloud forest approximately twenty five years in age. We examined size and distribution of individual trees in each transect. With this data, we intend to demonstrate trends in forest structure typical of primary and secondary forests.

Methods

The cloud forest surrounding Cerro de la Muerte, Costa Rica, contains adjacent patches of primary and secondary forest. This makes it an ideal location to examine succession and growth patterns of regenerating forest.

We compared two transects in differing forest types. The first transect was in a primary cloud forest near Cerro de la Muerte, Costa Rica, on October 19, 1998. It was oriented in an east-west direction perpendicular to a ridge, 50 m down each slope for a total length of 100 m. The second transect, completed the next day, was 50 m long, and was also oriented east to west in a nearby area of secondary forest. The transects were 2 m in width (1m on each side of the central transect line). All trees with a diameter at breast height (DBH) equal to or greater than 2.5 cm were included in the study if at least half of their base was within the transect boundaries.

We made several different observations for each tree. We recorded the species of each individual and its DBH (to the nearest 0.1 cm). We determined the relative coordinates of each tree using the distance (to the nearest 0.01 m) from starting point and distance (to the nearest cm) from the center line of the transect. Tree height (to the nearest 0.25 m) was

visually estimated from the ground level to the highest point of the tree.

To classify the DBH distribution of the trees in the transects, we established the following six DBH classes: (1) 2.5 cm-5.0 cm; (2) 5.1 cm-10.0 cm; (3) 10.1 cm-20.0cm; (4) 20.1cm-40.0cm; (5) 40.1cm-80.0cm; and (6) 80.1cm-160.0cm. A logarithmic scale was used to account for large outlying values. The number of individuals within each class was plotted for each transect. To determine the relationship between tree height and DBH, a logarithmic regression was performed on plots of height vs. DBH for the primary and secondary forest transects.

Plots of tree height vs. distance along each transect were generated to demonstrate geographic trends in primary and secondary forest architecture. To determine the number of individuals per hectare, tree densities were calculated by dividing the number of trees in one transect by 0.02 ha or 0.01 ha, the area covered by the primary and secondary transects, respectively.

A frequency distribution of tree species was constructed for both forests to demonstrate patterns in species dominance. The Shannon-Weiner index was used to determine the species diversity and evenness for our transects. To generate a species area curve, the area (in m²) surveyed in each transect prior to encountering the first individual of each species was calculated. These values are equal to the product of the position (in m) along the transect of the tree in question and the transect width (2 m). The values were compiled into two species area curves, one for each forest type. Each set of data was subjected to a regression analysis. The best fit curve of the form $s = cA^z$ was

determined where s = the number of species, A = area, and c and z are constants.

All statistical analyses were performed using the *JMP* statistical package (Sall and Lehman 1996).

Results

In our primary forest tract the greatest number of individuals fell within the lowest DBH class (2.5 cm- 5.0 cm; Figure 1). Numbers of individuals decreased with increasing DBH class (Figure 1). In the secondary forest the greatest number of individuals were in the second DBH class (5.1 cm-10.0 cm; Figure 2). Beginning with the third DBH class (10.1 cm- 20.0 cm) there was a sharp decrease in number of individuals found (Figure 2).

Maximum, minimum and average values for DBH and height in each forest transect are given in Table 1. DBH did not differ between the two types of forest (t -test: $n = 71$; $t = 1.487$; $P = 0.142$). However, tree height was greater in the primary forest than in the secondary forest transect (t -test: $n = 71$; $t = 2.057$; $P < 0.043$). When a logarithmic regression was performed on each plot of height vs. DBH, variance in primary forest tree height was explained well by DBH ($n = 31$; $R^2 = 0.82$; $P < 0.001$; Figure 3), while DBH did not explain variance in height of secondary forest trees ($n = 40$; $R^2 = 0.53$; $P < 0.001$; Figure 4). Additionally, in the primary forest transect 75% of all *Quercus costaricensis* individuals were greater than 10 m in height (Figure 3). In contrast, all *Q. costaricensis* in the secondary forest were less than 10 m in height. Furthermore, with the exception of one emergent tree, the secondary forest canopy height appeared level at approximately 10 m (Figure 4).

Graphic representation of height vs. distance for primary forest indicated a geographic trend in growth patterns. Trees were short and densely clustered toward the bottom of the eastern slope and thin out toward the ridge (Figure 5). On the western slope trees were considerably higher and more evenly spaced (Figure 5). The height vs. distance graph for secondary forest revealed dense clusters of relatively short trees interspersed with gaps of little to no growth (Figure 6).

Quercus costaricensis was the dominant species in both primary and secondary forest but was notably more dominant in the secondary forest transect (Figure 7). Ten tree species were represented in the primary forest transect while the secondary forest transect contained eight species. Results of Shannon-Weiner analyses revealed greater species diversity and higher species evenness in the primary forest transect (Table 2).

The species area curves indicated that species accumulated more rapidly in the primary forest than in the secondary forest. Species in both forests accumulated in an exponential manner (Figures 8, 9). The species area curve for the primary forest, however, appeared linear (Figure 8), but this was probably because it did not begin to level off within the size of area we sampled.

Discussion

We observed a number of differences in forest structure and species composition between the primary and secondary forest transects.

Numerically, both transects were dominated by trees in the smallest DBH and height classes. However, the two transects

differed in that the primary forest contained several trees that were very large both in terms of DBH and height. Such trees were not present in the secondary forest. Furthermore, the relationship between height and DBH indicated that the secondary forest was dominated by relatively thin trees. This was expected of successional forests, where tree competition for light renders investment in height more likely than investment in increasing stem diameter. Conversely, light may not be as limiting for established canopy trees in the primary forest, enabling additional investment in stem diameter or buttressing for greater stability.

The correlation between height and DBH was much stronger in the primary forest than in the secondary forest. This could indicate that growth rates of individual trees were highly similar in the primary forest, either because the trees were of the same species, or due to similar growth patterns between species. In contrast, the weaker correlation between height and DBH in the secondary forest indicates a diversity of tree growth rates and patterns.

The difference in dominant DBH classes between the two transects could be explained by differing recruitment patterns. The smallest DBH class in the secondary forest was not as dominant as the next largest DBH class. This could have been the result of a period of high recruitment which most likely occurred when the forest was allowed to regenerate. The presence of numerous small, dead trees in the secondary transect suggests that seedling recruitment continually occurs, but that most of these saplings were not able to survive in the shade of the larger, established trees.

Shade may also be a barrier to recruitment in the primary forest. However,

most of the shade is cast by a few very large canopy trees rather than numerous smaller trees as in the secondary transect. Our data indicated that the secondary forest appeared to have a more closed canopy than the primary area. This, however, is misleading. The trunks of canopy trees in the primary forest were widely separated. There was a distinct canopy over the primary transect, but the trunks of these trees were often rooted outside of the transect. Similarly, apparent differences in the tree height between east and west slopes of the primary transect are misleading due to small sample size.

Both transects were dominated by *Quercus costaricensis*. The domination of the secondary canopy by small *Q. costaricensis* suggests that it will mature into a forest resembling our primary tract. The species-area curves for both transects

resemble the theoretical species-area relationship. However, the curves do not appear to be approaching an asymptote. Clearly, larger transects are needed to accurately characterize the species composition of the forests. The size of these transects cannot be calculated as the total areas of the forests are not known.

Both evenness and diversity were greater in the primary forest. This is contrary to the conventional view of high altitude oak forest dynamics (Maarten Kapelle, personal communication). Sampling error due to small transects most likely accounted for these differences.

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Table 1a. Maximum, minimum, mean and standard deviation of Diameter at Breast Height (DBH) in cm for forests near Villa Mills, Cerro de la Muerte, Costa Rica.

	Max. DBH	Min. DBH	Mean DBH	Std. Dev.
Primary forest (n=31)	115.0	2.5	13.24	21.10
Secondary forest (n=40)	23.9	2.7	8.15	4.42

1b. Maximum, minimum, mean and standard deviation of tree height in m for forests near Villa Mills, Cerro de la Muerte, Costa Rica.

	Max. Height	Min. Height	Avg. Height	Std. Dev.
Primary forest (n=31)	35.0	2.25	8.97	7.90
Secondary forest (n=40)	12.5	2.0	6.23	2.63

Table 2. Species diversity (H') and evenness (J) for forests near Villa Mills, Cerro de la Muerte, Costa Rica.

	H'	H'_{\max}	J
Primary forest (n=31)	0.91	1.04	0.875
Secondary forest (n=40)	0.559	0.903	0.619

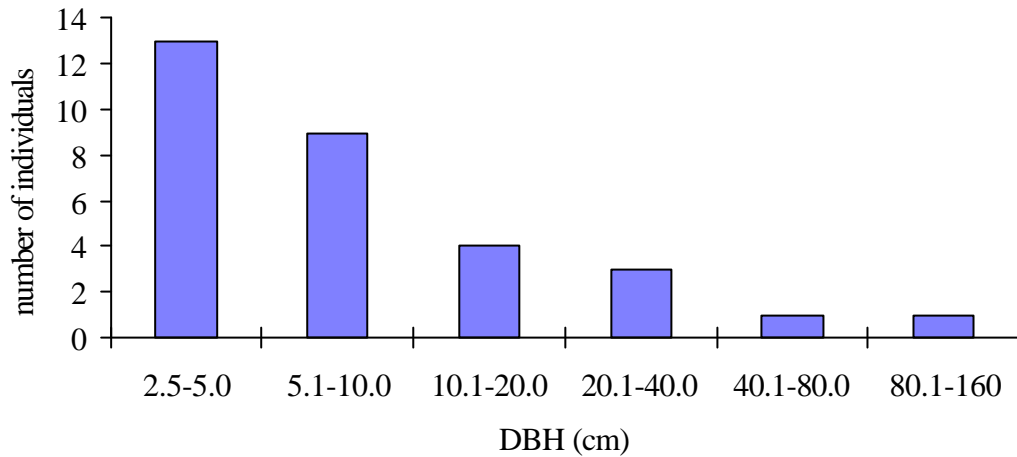


Figure 1. The number of trees found in primary forest plotted against 6 classes of diameter base heights. Forest was located near Villa Mills, Cerro de la Muerte, Costa Rica.

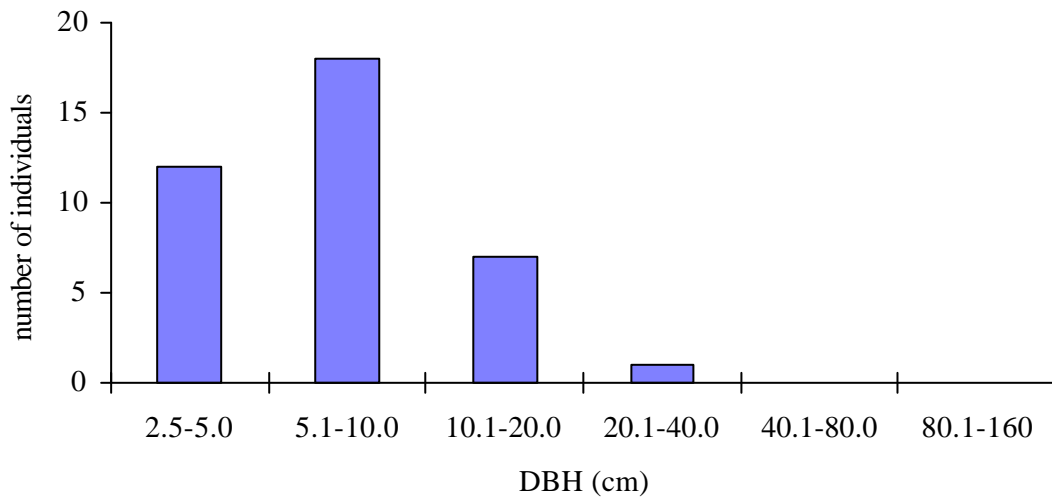


Figure 2. The number of trees found in the secondary forest plotted against 6 classes of diameter base heights. Forest was located near Villa Mills, Cerro de la Muerte, Costa Rica.

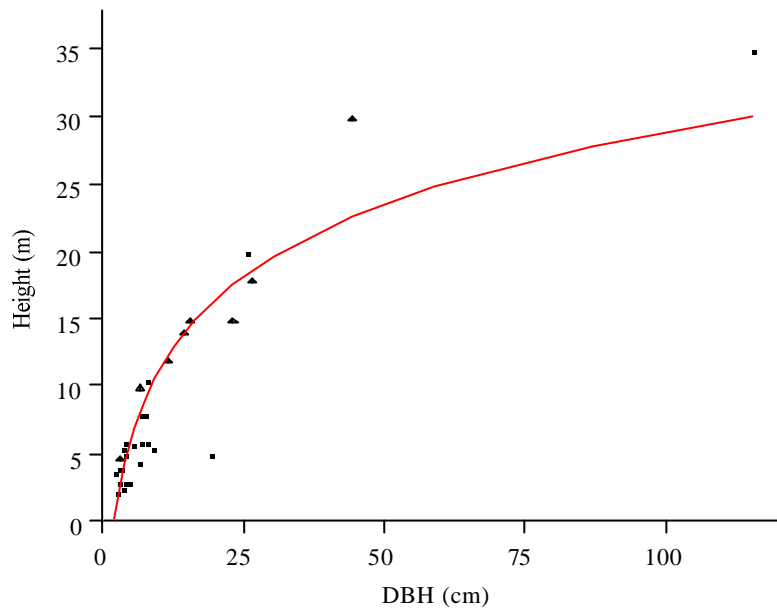


Figure 3. Height vs. Diameter at Breast Height (DBH) for a transect in primary forest near Villa Mills, Cerro de la Muerte, Costa Rica. The curve represents a logarithmic regression (Height = $-7.0056 + 7.81542 \log(\text{DBH})$). Triangular data points represent individuals of *Quercus costaricensis*. Square data points represent individuals of all other species found.

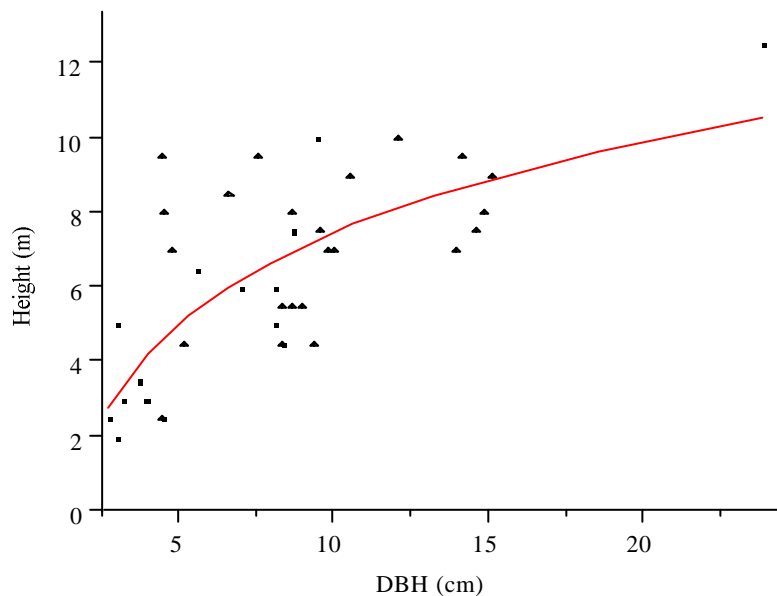


Figure 4. Height vs. Diameter at Breast Height (DBH) for a transect of secondary forest near Villa Mills, Cerro de la Muerte, Costa Rica. The curve represents a logarithmic regression (Height = $-0.7378 + 3.55211 \log(\text{DBH})$). Triangular data points represent individuals of *Quercus costaricensis*. Square data points represent all other species found.

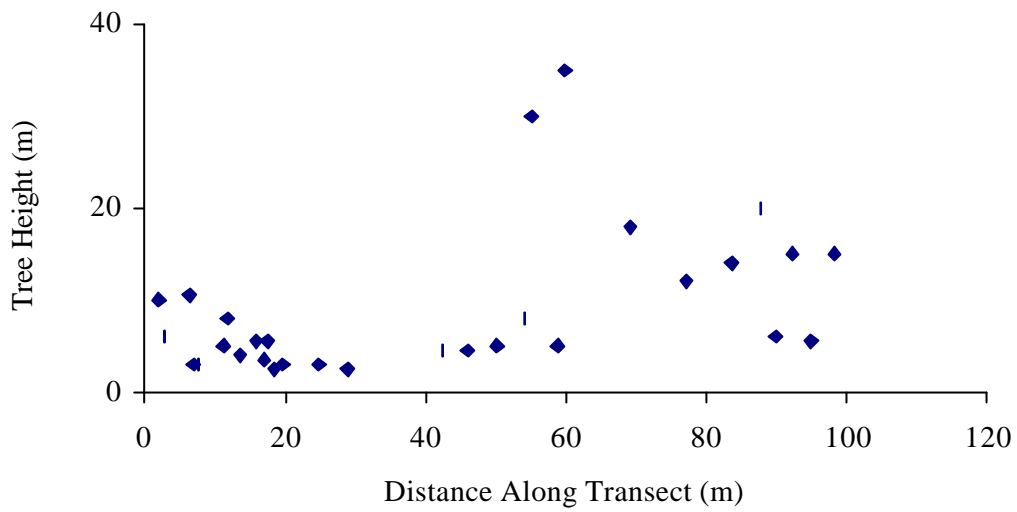


Figure 5. Tree height vs. distance along transect in a primary forest near Villa Mills, Cerro de la Muerte, Costa Rica.

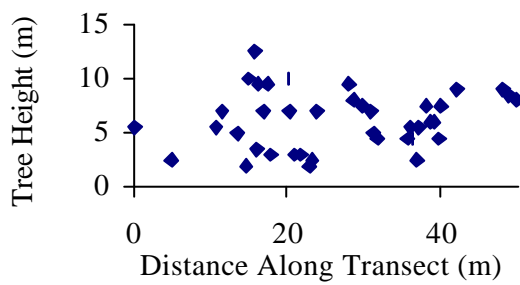


Figure 6. Tree height vs. distance along transect in a secondary forest near Villa Mills, Cerro de la Muerte, Costa Rica.

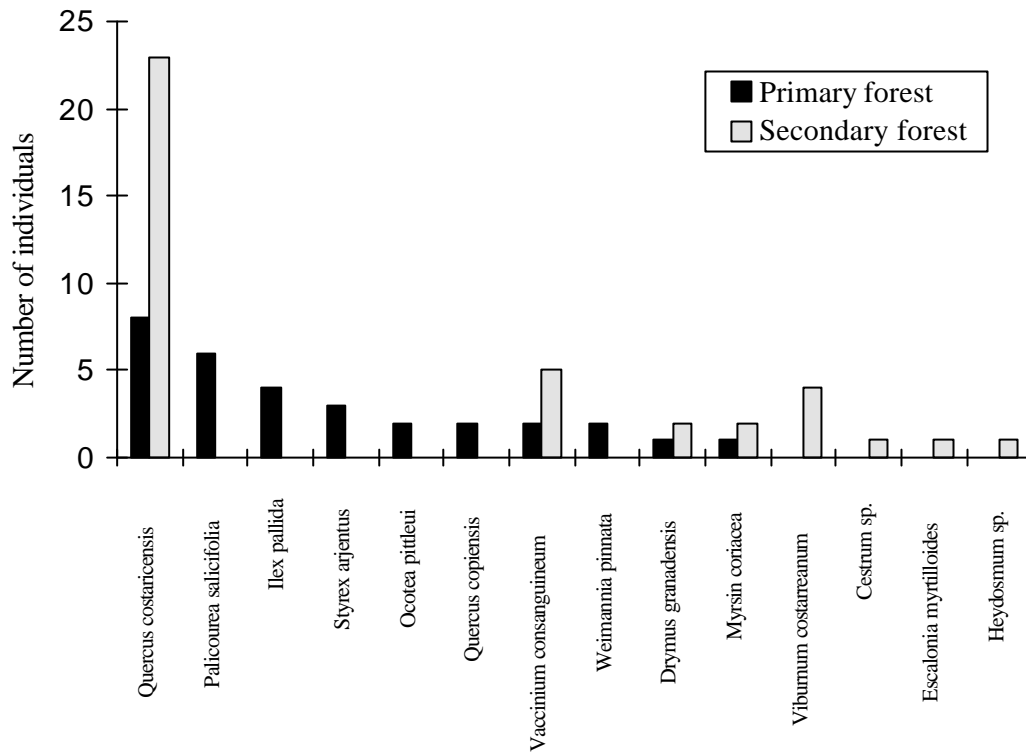


Figure 7. Number of individuals for each tree species encountered in primary and secondary forest transects near Villa Mills, Cerro de la Muerte, Costa Rica.

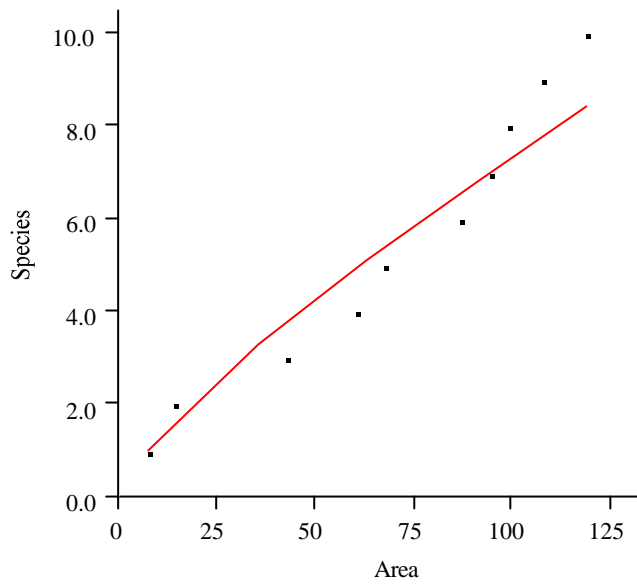


Figure 8. Number of species vs. area (m^2) for primary forest near Villa Mills, Cerro de la Muerte, Costa Rica. Curve represents exponential regression. (Species = $(0.0235) \text{Area}^{0.786}$, $R^2 = 0.956$, $n = 9$)

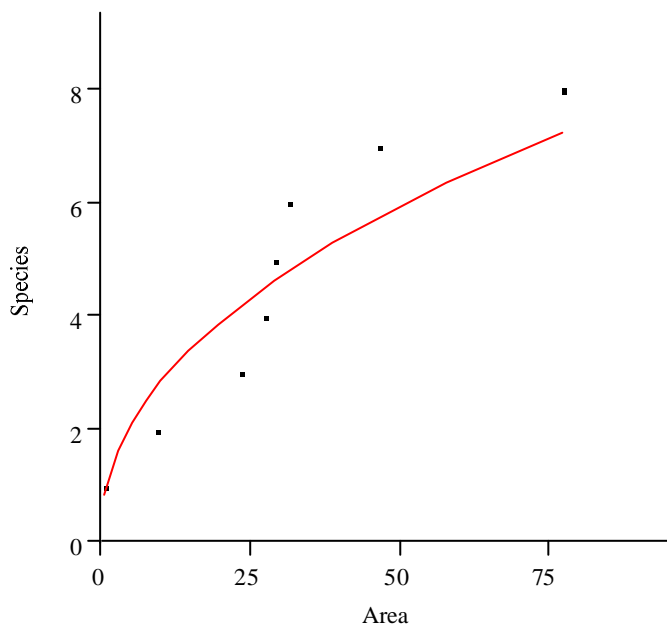


Figure 9. Number of species vs. area (m^2) for secondary forest near Villa Mills, Cerro de la Muerte, Costa Rica. Curve represents an exponential regression. (Species = $(0.924) \text{Area}^{0.464}$, $R^2 = 0.897$, $n = 8$)

PALO VERDE

Effects of leaf age on extra-floral nectar production and ant activity in *Acacia collinsii*
(Mimosaceae)

Category: Independent Project
Participants: Athena T. Dodd; Dr. Christopher Ivey (resource person)
Site: Palo Verde
Key words: *Acacia collinsii*, extrafloral nectaries, herbivore defense, nectar production

Introduction

Herbivory is a common threat to plant health and plants have responded with a variety of defense strategies. One example of anti-herbivore defense is the ant-acacia mutualism. Several species of *Acacia* (Mimosaceae) are found in association with ants of the genus *Pseudomyrmex*. The ants live in hollow thorns and feed from extra-floral nectaries on leaf petioles and protein-rich Beltian bodies on the tips of new leaflets (Janzen 1983a). The ants commonly clear the surrounding ground of competing vegetation and protect the *Acacia* from most arthropod herbivores, including various beetles and lepidopteran larvae (Janzen 1983a, Cronin 1993).

Leaf age may be an important factor in herbivore attack because herbivores such as the Ant-Acacia Beetle, *Pelidnota punctulata*, have been observed to preferentially feed on young leaves (Janzen 1983b). Young leaves lost to herbivory can be considered resource sinks due to lost biomass and unfulfilled photosynthetic potential. Consequently, it should be advantageous for *Acacia* trees to combat young-leaf herbivory. *Acacia* nectaries are located on each leaf petiole, thus it seems possible that nectar production could be locally adjusted to influence ant presence. Such a phenomenon has already been described in

the ant-protected legume, *Inga oerstediana*, which secretes extra-floral nectar only during the period of leaf maturation (Koptur 1983). I hypothesized that extra-floral nectar secretion in *Acacia collinsii* parallels this pattern and expected to observe higher nectar production and ant activity in young leaves than in old leaves. To test my predictions, I measured nectar volume and concentration and calculated the amount of sugar produced by nectaries of young and old leaves of *A. collinsii*. To gauge ant response to nectar availability, the amount of time spent by ants at extra-floral nectaries in the two leaf types was also observed.

Materials and Methods

This study was conducted on 67 November 1998, in seasonal dry tropical forest at Palo Verde National Park, Costa Rica (see Hartshorn 1983 for detailed site description). Twelve *Acacia collinsii* trees between two and three meters tall were chosen near the Palo Verde field station road and airstrip. Light-green leaf coloration was used as an indication of youth in selecting one young and one old leaf from each tree. Young leaves with persisting Beltian bodies were not included. Pairs of leaves were chosen on a given tree such that the number of nectaries per petiole was constant and this number was recorded along with rachis length to control

for their effects on nectar production. Ants were excluded from the nectaries using Tangle-Trap insect-trapping glue (Tanglefoot Co., Grand Rapids, Michigan) applied around the stem above the nectaries, followed by manual removal of ants remaining on the leaf. Bags of insect netting enclosed leaves and petioles to exclude any flying visitors to the nectaries. Leaves were removed from the tree between two and four hours after bagging to obtain nectar measurements.

Nectar was collected and measured in 1 μL capillary tubes to 1/32 μL accuracy. Sugar concentration (% wt/vol) was measured using a sugar refractometer (Model No. 300010, Sper Scientific Ltd., Scottsdale, Arizona). Amount of sugar (μg) was calculated as the product of nectar volume and concentration. Estimates of sugar concentration were increasingly variable and possibly unreliable with minute nectar volumes, so secretions of less than 1/16 μL were excluded from all analyses comparing nectar concentration.

Ant activity was observed in young and old leaves of ten different trees along the road and airstrip. Activity was measured as the number of seconds spent by each ant at the extra-floral nectaries during a five-minute interval. Nectary number was held constant for leaves within a tree.

Statistical analyses were performed using Jmp 3.2.2 (SAS Institute, Inc., 1989). To avoid inadvertent weighting of plants with high mean values in the analyses, raw data for nectar and ant variables were transformed into relative differences (RD) according to the formula: $\text{RD} = (\text{young} - \text{old}) / (\text{young} + \text{old})$. Because of the small sample sizes involved, differences between leaf categories were analyzed using a paired

Wilcoxon signed-rank tests. In addition, Pearson correlation coefficients were calculated between nectar and leaf dimension variables.

Results

Mean nectar volume, sugar concentration, amount of sugar, and ant visitation tended to be higher in young leaves, but these differences were not significant (Table 1). Analyses of the relative differences revealed that young leaves in this study had a higher percentage of the total amount of sugar collected from each tree (Table 2). Relative nectar volume and sugar concentration however, did not vary significantly between leaf types (Table 2).

Amount of sugar was not correlated with the number of nectaries on the petiole ($r = -0.3148$, $P = 0.2350$) nor with rachis length ($r = -0.2879$, $P = 0.2957$).

Discussion

Contrary to my hypotheses, new leaves did not produce more or richer nectar. The evenness of ant activity across leaf categories also contradicts my predictions. Thus, one study's observations that young *Acacia* leaves experience less herbivory than old leaves (Knoll et al. 1998) cannot be explained by the influence of extra-floral nectar secretion on ant distribution. Instead, the discrepancy may be explained by the presence of non-renewable Beltian bodies that are found only on new leaves. Another ant-mutualist, *Cecropia spp.*, produces renewable food bodies at higher rates on young leaves than on old leaves, accounting for increased ant-defense on young leaves (Louie LaPierre, pers. comm.). To confirm this for *A. collinsii*, data would have to be collected

on ant-visitation over different availabilities of Beltian bodies.

The transformed variables revealed differences in the amount of sugar released by different-aged leaves. Relative to the total amount of sugar that was measured from each tree, nectaries on young leaves secreted more sugar than those on old leaves. The amount of sugar produced in each leaf does not appear to be influenced by the number of nectaries or on the size of the leaf. Because sugar is an energy-rich molecule that is produced at a cost to the plant, the trees in this study can be said to have invested more energy in young leaves than in old leaves due to the amount of sugar exuded in their nectar. However, these differences were not reflected in my observations of ant-visitation. Perhaps *Pseudomyrmex* ants respond to nectar availability and quality on a whole-plant basis rather than a branch-by-branch basis.

Acknowledgments

This project would not have been possible without the patient encouragement and enthusiastic field assistance of Chris Ivey. Investigation into ant activity was performed in collaboration with Lisa Stano. Todd Shelley helped too, even though it made him late for lunch.

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Table 1. Means and standard errors for nectar attributes and ant visitation to extra-floral nectaries of *Acacia collinsii*. Nectar samples were collected from twelve trees in Palo Verde National Park, Costa Rica, after 2-4 hr of insect exclusion.

	Young Leaves	Old Leaves	<i>n</i>	<i>P</i>
Nectar Volume (μL)	0.4636 ± 0.1551	0.2656 ± 0.0961	24	0.156 ^a
Percent Sugar Content (wt/vol)	73.8 ± 5.3	64.0 ± 7.8	12	0.563 ^a
Amount Sugar (μg)	44.0 ± 12.3	24.4 ± 12.3	12	0.469 ^a
Ant visitation (ant secs/5 min.)	101 ± 37	98 ± 43	20	0.8755 ^b

^a paired Wilcoxon signed-rank test

^b paired T-test

Table 2. Mean relative differences (% RD = (young - old) / (young + old)) between young and old leaves of *Acacia collinsii* in extra-floral nectar attributes^a and ant activity. Samples were collected from twelve trees in Palo Verde National Park, Costa Rica.

	Mean RD \pm SE	<i>n</i>	<i>T</i>	<i>P</i>
Nectar Volume	10.5 ± 15.7	12	0.6652 ^b	0.5210
Percent Sugar Content	6.8 ± 7.0	6	0.9725 ^b	0.3755
Amount of Sugar in Nectar	56.3 ± 6.8	6	8.2747 ^b	0.0004
Ant Visitation Time	17.6 ± 16.6	10	1.0607 ^b	0.3165

^a Nectar production measured after 2-4 hrs of exclusion by bagging.

^b paired Wilcoxon signed-rank test with *n* = number of pairs. Only samples with volumes exceeding 0.0625 μL were included in comparisons.

Acacia collinsii size requirements for colonization by *Pseudomyrmex*

Category: Independent Research Project
Participants: Margaret Yu and Naamal De Silva
Site: Palo Verde
Key words: *Acacia collinsii*, colonization, *Pseudomyrmex*

Introduction

Several species of *Acacia* trees have a mutualistic relationship with *Pseudomyrmex* ants. *Acacia* trees provide the ants with shelter in its hollow thorns and food from extrafloral nectaries. In addition, the trees produce Beltian bodies, which offer a source of amino acids and lipids (Kricher 1997). In return for shelter and nutrition, the ants aggressively deter herbivory. They have been known to prune and kill neighboring plants, giving the *Acacia* tree adequate amounts of light and creating a circle of cleared vegetation around it.

For the *Acacia-Pseudomyrmex* mutualism to function, the *Acacia* tree must have sufficient ant attractants to support an ant colony. Its ability to meet the needs of *Pseudomyrmex* may be limited by its size. We predicted that *Pseudomyrmex* requires a minimum *Acacia* size to colonize the tree, and we expected to find that the smaller trees would be uncolonized, whereas larger *Acacia* trees would be colonized. Since ant presence deters herbivory, we also predicted that larger trees would exhibit lower amounts of herbivory.

Methods

The study was conducted at the Palo Verde Biological Field Station in Costa Rica, on November 5-6, 1998. *Acacia collinsii* was chosen as the study organism because of its abundance at Palo

Verde. *Acacia collinsii* is characterized by red thorns, yellow Beltian bodies, and round, petiolar nectaries clustered in groups of 1-4. It is colonized most often by *Pseudomyrmex ferruginea*, but also by *P. belti* and *P. nigrocincta*.

The following five measurements of size were taken for 60 trees (primarily < 1.5 m tall) found on the OTS air strip at Palo Verde: height, maximum crown width, number of mature thorns, number of nectaries, and number of Beltian bodies. The presence or absence of *Pseudomyrmex* colonization, including the species, was noted for each tree. For trees ≥ 1.5 m tall, a height approximation and maximum crown width measurement was made; presence of ant colonization, thorns, nectaries, and Beltian bodies was noted but not quantified. For all trees, it was noted whether the individual was found growing alone or in a conspecific clump. Clumped individuals were defined as either growing in the same circle of cleared vegetation with other trees, or close enough to another *A. collinsii* that their branches were touching.

To measure herbivory, 20 leaves per tree were randomly selected and placed in "high", "low", or "no" herbivory categories. Completely intact leaves were categorized as "no herbivory", overall intact leaves with a few missing or damaged leaflets were categorized as "low herbivory", and leaves with large, noticeably damaged patches were

categorized as “high herbivory”. For small trees with fewer than 20 leaves, all leaves were categorized. The percentage of leaves in each category was calculated by taking the number of leaves in the category and dividing it by the total number of leaves sampled. Herbivory scores were assigned to each tree by using the formula, $H = 2(\% \text{ high herbivory}) + 1(\% \text{ low herbivory}) + 0(\% \text{ no herbivory})$.

All statistical analyses were performed using the computer software JMP 3.2.2. (Sall and Lehman 1996). To verify that each measurement was an indicator of size, linear correlation tests were done between height and each of the other measurements of tree size (maximum crown width, number of thorns, number of nectaries, and number of Beltian bodies). We found strong positive correlations (see Results) so we used tree height to indicate tree size for subsequent analyses. The data were separated into two categories based upon ant presence, and *t*-tests were done to compare height. First the *t*-test was done using all of the data, to test the null hypothesis of no height difference between *Acacia* trees with and without ants. However, since only a few of the individuals were > 1 m tall, and all of them were colonized (see Results), these few mature trees could have skewed the mean for the colonized group, so we repeated the *t*-test using only the data from individuals < 1 m tall. We also noticed a difference in colonization patterns between the trees that were growing individually or in a clump (see Results), so we repeated the *t*-test using data only from individuals found growing in a conspecific clump. Next, a *t*-test was done to determine if there was a significant difference between the herbivory scores of the two groups, to verify that ant presence

reduces herbivory. Finally, a linear correlation test was run between height and herbivory scores, which in combination with the last test, would test the null hypothesis of no difference in herbivory between large and small trees.

Results

The majority of *A. collinsii* were small trees, which were characterized by heights of < 1 m tall ($n = 49$), maximum crown widths of < 1 m ($n = 51$), and few mature thorns and nectaries (Table 1). *Acacia collinsii* were found growing in isolation as well as in clumps around a parent tree. All trees ≥ 1.5 m tall had been colonized by *Pseudomyrmex*, and often grew within a circle of cleared vegetation that encompassed other trees. Trees < 1 m in height that were found growing in a clump were either colonized or uncolonized, whereas trees < 1 m found growing in isolation were all uncolonized, although some trees had holes and larvae in their thorns. The smallest colonized individual was 0.17 m in height, and was found growing in a clump. The largest uncolonized individual was 0.75 m in height, and was found growing in isolation. The colonized individuals were occupied predominately by *P. ferruginea*, but *P. belti* and *P. nigrocincta* were also observed.

Strong positive linear correlations were found between height and each of the following: maximum crown width, number of thorns, and number of nectaries (Table 2). No correlation was found between height and the number of Beltian bodies. Ant-colonized trees were significantly taller than uncolonized trees (Table 3). This result was confirmed by the *t*-test using all of the data. Since there were a few

individuals > 1 m tall ($n = 11$) that could have exaggerated the difference between the two groups, the t -test was done again without those individuals (see Methods), which still produced a significant difference. The t -test was repeated once more with only the individuals growing in a clump because none of the isolated individuals < 1 m tall were colonized. This seemed to be because of different minimum tree size requirements by *Pseudomyrmex* if the tree was in a clump or not, and we did not want to compare clumped individuals with isolated individuals (see Discussion). Again, there was still a significant height difference. Herbivory was significantly lower in ant-colonized trees (Table 4), and had a significant but weak negative correlation with height (Table 2).

Discussion

Although we found that colonized *A. collinsii* were taller than uncolonized *A. collinsii*, we did not find a minimum height that was required in order for a tree to support an ant colony. Since height was strongly correlated with our other size measurements of maximum crown width, number of thorns, and number of nectaries, we concluded that we did not find a minimum size of *A. collinsii* required by *Pseudomyrmex* for colonization. Thus we can not reject the null hypothesis that *Pseudomyrmex* need a minimum size of *Acacia* at this time.

However, the size of an individual *A. collinsii* may not be important if it is in a clump of trees. According to Janzen (1983), a single *Pseudomyrmex* colony can colonize all of the *Acacia* shoots in one clump, which may contain up to 30 individuals. The ants are thus treating all of the trees in the clump as one individual,

since all the trees support only one colony. This makes comparing individual trees problematic since some individuals may be colonized because it is part of a clump, and the clump's large size is sufficient to support the ant colony, whereas by itself the individual tree may not be large enough. Thus, sampling isolated individuals along with individuals growing in a clump is not a consistent way of measuring *A. collinsii* size. Therefore, trees growing in a clump should not be treated as separate individuals, and the relevant data is the total clump size. Measuring the size of a clump could be difficult, but this could be avoided by only collecting measurements of isolated trees. Even though our study focused on trees < 1 m in height, since none of the isolated trees were colonized, a study focusing on larger isolated trees may find a minimum size for colonization.

Although all the isolated trees were uncolonized, some had holes in their thorns. These may have been abandoned attempts at colonization, for living larvae were sometimes found inside the thorns. If this were the case, then it could suggest that those trees were unable to provide for the colony, possibly due to its inadequate size. Identification of the larvae as *Pseudomyrmex* and long-term observational studies to discover the source of these holes would be useful.

We found that herbivory was lower on taller trees, although the correlation was rather weak. We hypothesized that herbivory would be lower on tall trees because tall trees tended to be colonized and protected by ants. Although it is possible that herbivory was lower because tall trees are physically less accessible to herbivores, this was already tested by Janzen, who sprayed *Acacia* trees with

pesticides to eliminate the ants, and found that *Acacia* trees without ants exhibited significantly higher amounts of herbivory than the *Acacia* trees with ants (Janzen 1966, cited in Kricher 1997). Thus, based upon his study, this hypothesis may be discarded in favor of the original explanation.

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Table 1. Size measurements and herbivory scores for *Acacia collinsii*, Palo Verde National Park, Costa Rica. Upper limits of number of thorns and number of nectaries were not quantified. Herbivory scores have a possible range from 0-2 (see Methods for calculation).

	<i>n</i>	minimum	maximum	mean	median	SD
Height	60	0.09 m	4 m	0.73 m	0.35 m	0.98 m
Max. crown width	60	0.06 m	3.8 m	0.53 m	0.21 m	0.83 m
# of thorns	53	0	≥ 264	25.5	6	54.8
# of nectaries	53	0	≥ 461	48.4	16	89.1
# of Beltian bodies	56	0	584	48.6	0	113.9
Herbivory score	59	0.5	2	1.5	1.5	0.43

Table 2. Results of linear correlation tests of data collected from *Acacia collinsii* at Palo Verde National Park, Costa Rica.

	<i>n</i>	<i>r</i>	<i>P</i>
Height vs. Maximum crown width	60	0.9087	< 0.0001
Height vs. # of thorns	53	0.9699	< 0.0001
Height vs. # of nectaries	53	0.8661	< 0.0001
Height vs. # of Beltian bodies	56	0.1617	0.2339
Height vs. Herbivory score	59	-0.3584	0.0053

Table 3. Results of *t*-tests comparing tree height of *Pseudomyrmex*-colonized and uncolonized *Acacia collinsii* at Palo Verde National Park, Costa Rica. The *t*-test compared two subsets of the data in addition to the full data set (see Methods).

	<i>n</i>	mean height (m)	standard error	<i>t</i>	d.f.	<i>P</i>
all data	60			-4.581	58	< 0.0001
uncolonized	33	0.2818	0.1469			
colonized	27	1.2848	0.1624			
height < 1 m	49			-2.543	47	0.0144
uncolonized	33	0.2818	0.0281			
colonized	16	0.4069	0.0404			
clumped trees	26			-5.255	24	< 0.0001
uncolonized	11	0.1864	0.0329			
colonized	15	0.4140	0.0282			

Table 4. Comparison of herbivory level of *Pseudomyrmex*-colonized and uncolonized *Acacia collinsii* at Palo Verde, Costa Rica. Up to 20 random leaves were selected and classified as high, low, or no herbivory (see Methods for criteria and herbivory score calculation). *t*-test results comparing overall herbivory are shown below the table.

	Colonized	Uncolonized
<i>n</i>	26	33
% high herbivory	0.456	0.763
% low herbivory	0.478	0.140
% no herbivory	0.066	0.097
mean herbivory score (H)	1.297	1.667

n = 59

t = 3.592

P = 0.0007

How plant and insect diversity are affected by *Typha dominguensis* and *Parkinsonia aculeata* in Palo Verde National Park, Costa Rica.

Category: Independent Project
Participants: Jessica Lynch, Jennifer Havlik, and Serena Black
Site: Palo Verde
Key words: Aquatic insects, diversity, *Parkinsonia aculeata*, *Typha dominguensis*, wetlands

Introduction:

Species diversity is a measure of the composition of a community that takes into account species richness (number of species) and abundance patterns (Begon et al. 1996). An area with one dominant species and many rare species is expected to have lower diversity than an area with many common species (Meffe & Carroll. 1997). Consequently, invading dominant species can often cause a decrease in local diversity (Meffe & Carroll. 1997).

Aquatic plants provide food and shelter for many aquatic insects. Certain insects rely on specific plant species and cannot survive without them (Borror et al. 1989). A change in the composition of plant species would therefore be expected to affect the insect community. It is possible that a decrease in plant diversity within a given area would favor generalist insects and lead to a decrease in insect diversity.

Wetlands are an important habitat in Palo Verde National Park in Costa Rica. In 1920, intensive cattle ranching began in these marsh areas. In 1977, the wetlands in the area were recognized as an important habitat for waterfowl and a national wildlife refuge was created. As a result, cattle ranching was stopped in the area in 1981. At that time, two opportunistic species, *Typha dominguensis* and *Parkinsonia*

aculeata, started invading the marsh. By 1986 *T. dominguensis* had essentially taken over the marsh, and the coastal birds that the refuge was created to protect no longer had a suitable habitat. Consequently, government-approved cattle grazing was initiated in 1987 to stop the spread of *T. dominguensis* and increase bird diversity in the area (Gill. 1988).

We studied how *Typha dominguensis* and *Parkinsonia aculeata* affect plant and insect diversity in the wetlands of Palo Verde National Park. We predicted that areas dominated by *Typha dominguensis* and *Parkinsonia aculeata* would exhibit lower plant and insect diversity than the open marsh.

Methods:

Typha dominguensis is a species of cattail that is found in shallow freshwater habitats (Heywood 1993). It is not native to the Palo Verde region (Eugenio Gonzales personal communication). *Parkinsonia aculeata* is a spiny tree that is also known as the Palo Verde tree (Gentry 1996). It is native to the dry forest of Palo Verde but has recently begun encroaching on the wetlands (Eugenio Gonzales personal communication). Both species grow densely in the areas where they have invaded the marsh.

We studied three 5 x 5 m plots in the grazed area of the marsh adjacent to the airstrip at the OTS field station in Palo Verde National Park. We performed the study November 5 and 6, 1998. We first surveyed the marsh from the bird tower and chose three areas for plots based on the presence or absence of *Typha dominguensis* and *Parkinsonia aculeata*. Plot I was in an open area 30 meters east of the bird tower and contained neither species. This area was characterized by thick mats of plants floating on the surface of the water. Plot II lay 50 meters south of the bird tower within a patch of *T. dominguensis*. Plot III was 20 meters southwest of the bird tower in an area dominated by *P. aculeata*. We studied plots in the interior of the *T. dominguensis* and *P. aculeata* patches to get an accurate estimate of diversity in the different habitats.

After marking the plots, we collected each plant species we encountered. We planned to count the number of individuals of each plant species within each plot. However, some species such as *Neptunia natans* formed an interconnected mat over the surface of the water, and we were unable to distinguish individual plants from above the water. Therefore, instead of counting the number of individuals, we made observations about the relative abundance and growth patterns of each of the species in the plot. We characterized each species as “very common”, “common”, or “uncommon” based.

We collected insects for 45 minutes at each plot. We used aquatic nets to capture the insects and kill jars filled with 60% alcohol solution to store them until they could be identified. We collected insects from just below the surface plant

layer and sampled from several randomly selected sites in each plot.

We also used a water chemistry test kit (LaMotte Limnology Test Kit Model AM-02, Chestertown, Maryland) to measure dissolved oxygen, pH, nitrate, and phosphate in samples of water we collected from each plot.

After we collected all of our specimens from the field, we identified plant and insect morphospecies. We also, insofar as possible, calculated the abundance of each type of insect. We calculate insect diversity (H') using the Shannon-Weiner index,

$$H' = \sum p_i \ln(p_i)$$

where p_i = fractional abundance of the i th insect group.

We also calculated species evenness (J) to account for the different number of insect groups that we found in each plot using,

$$J = H' / H_{\max}$$

where $H_{\max} = \ln(k)$ and k = # of insect groups.

Results:

We found 11 plant species in Plot I, 9 species in Plot II, and 10 species in Plot III (Table 1). In all three plots at least half of the species were unique (they were only found in one plot), and overall 73% of the plant species were only found in one plot (Table 1). Plot I had an even distribution of species between the three categories “very common” (27%), “common” (36%), and “uncommon” (36%). We classified six of nine species (67%) in Plot II as “common”. Six of ten species (60%) in Plot III were categorized as “uncommon” (Table 1).

In Plot I we collected 61 insect specimens belonging to 11 different morphological groups (Table 2). In Plot II we sampled 94 insects from 14 groups, and

in Plot III we collected 97 individuals from 12 groups. The dominant groups in Plot I were Odonata I (green and brown heads) and Coleoptera I. The dominant groups in Plot II were Hemiptera Belostomatidae (toe-biters) and Diptera (mosquito larvae). In Plot III the dominant groups were Odonata (green and brown heads) and Hemiptera Belostomatidae. (Table 2) The H' and J values for the three areas did not differ significantly (Table 3).

We found no difference in water quality between the three areas (Table 4).

Discussion:

We found no difference in insect diversity, plant diversity, or water quality between the three plots. Therefore, our hypotheses were not supported by the data that we collected.

We were unable to get a quantitative measure of plant species diversity because we did not have the equipment or expertise needed to count individuals of many species. We did, however, observe some qualitative differences in the relative abundance of plant species between the three plots. The *Parkinsonia aculeata* plot had a larger proportion of uncommon species than either of the other two plots. The water in that area had less surface plant cover than the other two areas. This may have been due to the fact that the *P. aculeata* trees were monopolizing the available sunlight. The *P. aculeata* plot was very clearly dominated by one plant species, and the rarity of other species in the plot suggested that some species might be excluded in an area more densely populated by *P. aculeata*.

Another interesting finding was that each plot had unique plant species. This suggests that the invasion of *Typha*

dominguensis or *Parkinsonia aculeata* into the marsh changes the composition of plant species. It is therefore possible that cattle grazing in the region is excluding other species in addition to *T. dominguensis*. Further research with a larger sample size is needed to determine if the different habitats do support different plant communities..

We observed very little difference in insect diversity among the three plots. It is possible that rainfall and tides may make it difficult for aquatic insects to live in one specific habitat, and the region may therefore support only habitat generalists. Also, many of the insects we collected were predacious which might be associated with high mobility. These insects might still be dependent on plants for shelter but they would not depend on any particular plant species for food. Further research needs to be conducted to determine the extent to which marsh insects depend on specific aquatic plants.

Another interesting observation was that we collected more mosquito larvae in the *T. dominguensis* habitat than in either of the other two plots. This suggests that mosquitos may prefer to lay their eggs in areas dominated by cattails. It is possible that the difference was skewed by the scale of our sample size. Collecting over a longer period of time and in multiple locations would be necessary to determine whether mosquitoes have any preference about where they lay their eggs.

More research needs to be performed with plots deeper within the *Typha dominguensis* and *Parkinsonia aculeata* patches in order to determine how these species affect the plant and insect diversity of the marsh. However, our results suggest that the invasion of *Typha dominguensis* and *Parkinsonia aculeata*

may affect plant species composition more than plant species diversity. Species composition is an important factor to consider when looking at species diversity. Often, the highest overall plant diversity is achieved when different habitats persist at intermediate levels within an area

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Table 1. Wetland plant species and their abundance in three plots at Palo Verde National Park, Costa Rica; dominant vegetation of the plots indicated in parentheses

	Plot I (open marsh)	Plot II (cattails)	Plot III (Palo Verde trees)
Almistaceae			
<i>Echinodorus andrieuxii</i>	NOT FOUND	NOT FOUND	Common
Araceae			
<i>Pistia stratioidis</i>	Uncommon	NOT FOUND	NOT FOUND
Cannaceae			
<i>Canna glauca</i>	Uncommon	NOT FOUND	NOT FOUND
Ceratophyllaceae			
<i>Ceratophyllum muricatum</i>	Common	NOT FOUND	NOT FOUND
Convolvulaceae			
<i>Aniseia martinicensis</i>	NOT FOUND	NOT FOUND	Uncommon
Cyperaceae			
<i>Cyperus articulatus</i>	Uncommon	NOT FOUND	NOT FOUND
<i>Fimbistylis</i> sp.	NOT FOUND	Uncommon	NOT FOUND
unidentified sp.	NOT FOUND	NOT FOUND	Uncommon
Fabaceae			
<i>Neptunia natans</i>	Very common	Common	Uncommon
<i>Parkinsonia aculeata</i>	NOT FOUND	NOT FOUND	Very common
Lemnaceae			
<i>Lemna gibba</i>	Very common	Very common	Uncommon
Lentibulariaceae			
<i>Utricularia gibba</i>	Uncommon	NOT FOUND	NOT FOUND
Marantaceae			
<i>Thalia gemiculata</i>	NOT FOUND	Common	NOT FOUND
Menyanthaceae			
<i>Nymphoides indicum</i>	Common	NOT FOUND	Common
Najadaceae			
<i>Najas guadalupensis</i>	NOT FOUND	Common	NOT FOUND
Nymphaeaceae			
<i>Nymphaea ampla</i>	Common	NOT FOUND	Common
Poaceae			
<i>Brachiaria mollis</i>	NOT FOUND	NOT FOUND	Uncommon
<i>Paspalum</i> sp..	Very common	NOT FOUND	NOT FOUND
<i>Penecitum</i> sp.	Common	Common	NOT FOUND
unidentified sp.	NOT FOUND	Common	NOT FOUND
Salviniaceae			
<i>Salvinia minima</i>	NOT FOUND	Common	NOT FOUND

Typhaceae			
<i>Typha dominguensis</i>	NOT FOUND	Very common	Uncommon

Table 2. Aquatic insect groups and their abundance in three marsh plots at Palo Verde National Park in Costa Rica; dominant vegetation of the plots indicated in parentheses

Insect group	Plot I (open marsh)	Plot II (cattails)	Plot III (Palo Verde trees)
Coleoptera I	12	9	6
Coleoptera II	0	1	5
Coleoptera III	2	0	0
Coleoptera IV ⇒ larvae	0	2	0
Coleoptera Dytiscidae ⇒ larvae	0	3	5
Coleoptera Dytiscidae ⇒ larvae with antennae	0	1	0
Diptera ⇒ mosquito larvae	8	21	0
Ephemeroptera ⇒ mayfly larvae	1	0	0
Hemiptera Belostomatidae	3	23	19
Hemiptera Gerridae	3	0	1
Hemiptera Naucoridae	3	15	12
Hemiptera Nepidae	1	5	1
Odonata I ⇒ black dragonfly nymph	9	1	4
Odonata II ⇒ striped dragonfly nymph	0	1	4
Odonata III ⇒ long abdomen dragonfly nymph	2	1	3
Odonata IV ⇒ green and brown dragonfly nymph	7	3	33
Odonata V ⇒ damselfly nymph	0	8	4
TOTALS	61	94	97

Table 3. Shannon-Weiner diversity indices and evenness values for three marsh plots at Palo Verde National Park, Costa Rica; dominant vegetation of the plots indicated in parentheses

	H'	H'max	J
Plot I (open marsh)	0.881	1.04	0.846
Plot II (cattails)	0.920	1.15	0.803
Plot III (Palo Verde trees)	0.877	1.08	0.813

Table 4. Water quality test results for three marsh plots at Palo Verde National Park, Costa Rica; dominant vegetation of the plots indicated in parentheses

Type of test	Plot I (open marsh)	Plot II (cattails)	Plot III (Palo Verde trees)
pH test	7	7	7
Phosphates (ppm)	<0.2	<0.2	<0.2
Nitrates (ppm)	<8.8	<8.8	<8.8
Dissolved oxygen (ppm)	1.8	1.4	1.2

Comparison of male and female behavior in *Jacana spinosa*

Category: Independent Project
Participants: Gregory Mitchell, Brad Feldman, Heather Fowler
Site: Palo Verde
Keywords: Animal behavior, *Jacana spinosa*, mating systems, polyandry, territoriality

Introduction

Polyandry is a mating system wherein one female mates with more than one male (Stephens 1979). One such organism that exhibits polyandry is the bird *Jacana spinosa*. Each female *J. spinosa* usually mates with 2–4 males and these polyandrous relationships last throughout the breeding season (Jenni 1983). Each male has a small breeding-feeding territory from which he excludes all other males, and each female has a super-territory encompassing 1–4 male territories, which she defends from all conspecific intruders (Jenni 1983). We examined whether the polyandry and territoriality of *J. spinosa* cause behavioral differences between males and females. We hypothesized that there would be differences in the amount of time that males and females spend in three behaviors. First, we hypothesized that females would spend more time foraging than males. Females usually lay successive clutches of 4 eggs, which can be laid every 7–10 days (Jenni 1983). We believed that females would forage more than males for food because of the physiological drain of developing and carrying the eggs. In addition, females are 75% heavier than males (Jenni 1983) and may need more food to maintain their larger body weight. Second, we hypothesized that females would spend more time defending territory than males because the female defends her super-territory, which comprises several

male territories, from both males and females. She therefore has the potential to be involved in more defensive interactions than the male, who defends his smaller territory only from other males. Third, we hypothesized that males spend more time sitting in one place because they incubate the eggs.

Methods and Materials

From a bird observation tower overlooking a marsh at Palo Verde National Park in Guanacaste Province, Costa Rica, we observed 18 individuals of *J. spinosa* during their breeding season. We observed them using binoculars for six hours, three hours in the morning and three hours in the afternoon: 4–5pm on 11/5; 7:30–9:30am, 3–5pm on 11/6; and 5:30–6:30am on 11/7. Each of us observed six birds for one hour each, for a total of 18 birds in 18 person-hours. The sample was evenly divided between males and females. Bird sex was determined by group consensus according to the bird's size, territorial range, and if the bird was seen mating, its position when mating; we assumed females to be larger birds with larger ranges, and to be beneath the male when mating. At hourly intervals we randomly selected males and females from the marsh area visible from the observation tower. We marked a time interval corresponding to the duration of one of seven different behaviors relating to

foraging, territory defense and sitting. Behaviors were defined as follows:

Pecking/Probing: moving beak down to substrate or using beak to penetrate substrate

Digging: sorting substrate with feet

Squawking: making noise (calling) while interacting with another bird

Displaying: raising wings above body

Sitting: resting in one place with legs folded beneath body and ventral surface of bird touching substrate surface

Mediating: female flying to break up 2 or more males interacting

Pecking attack: one bird pecking on another bird with its beak

The time intervals were 0–1 sec., 1–5 sec., 5–10 sec., 10–30 sec., 30 sec.–1 min., 1–2 min., 2–3 min., 3–4 min., and 4–5 min. If none of the listed behaviors were exhibited by the bird (e.g. the bird was standing and looking around), no behavior was marked. For each bird the marks for each time interval were summed for behaviors using the midpoints of the time intervals for each summation. Using the JMP statistical analysis program (SAS Institute Inc. 1996), t-tests were performed to test differences between males and females in the amount of time spent in each behavior. In addition to comparing the behaviors listed above, we combined some of the behaviors to form the larger behavioral categories of foraging and territory defense. We defined foraging as pecking/probing and digging. To test differences in time spent foraging we added together the time spent in those two behaviors. Likewise, we defined the components of territory defense as squawking, displaying, mediating, and pecking attacks and the time spent in each of these components were added together.

Results

Our data reveal no statistical difference between males and females in the amount of time spent foraging or defending territory (Table 1). There also was no difference between males and females in any single behavioral component of either foraging or territory defense (i.e. no difference in time spent pecking/probing, digging, displaying, etc.). There was, however, a statistically significant difference in the amount of time each sex spent sitting. Males spent more time sitting in one place than females.

Discussion

Our data suggest that there is no difference between males and females in the amount of time they spend foraging or defending their territory. It is possible that developing eggs do not noticeably drain the female physiologically because *J. spinosa* eggs are small relative to the body weight of the female. The average egg weighs 7.9g and the average female weight is 160.9g (Jenni 1983). Also, it has been suggested that females are more efficient than males at foraging. Stephens (1979) reports that the increased body weight of females allows them to overturn lily pads to search for insects, a technique that the lighter males are unable to do.

Our data also suggest that males and females spend the same amount of time defending territories despite differences in territory size and defense responsibility. We observed that it took time for a female to notice a male-male interaction and to fly the part of her territory where that interaction was occurring. In some cases, the males would cease interacting before the female arrived. This delay time is a possible

explanation for why the time spent in defense by males and females does not differ even though the female has the potential to be involved in more situations of territorial defense. In addition, males are submissive to females in social situations (Jenni 1983), and as such females may be more effective territorial defenders in interactions with males than males are with other males. This increased female defense efficiency may cause female-male interactions to be shorter than male-male interactions, reducing the total amount of time that females spend defending.

As expected, males spent more time sitting than females. This is most likely because they were sitting on their nests, incubating the eggs. Although we did not observe any nests, we did observe that when a single male sat repeatedly, he would return to sit in the same location on the marsh where he sat previously. From this observation we can infer that each male was returning to his nest to incubate the eggs. Thus, from our study, it appears that polyandry effects a difference in male and female *J. spinosa* behavior only in the amount of time spent sitting in one place and not in the amount of time spent foraging or defending territory. Because the average male : female ratio in *J. spinosa* is 2.3:1 (Jenni 1983), it is likely that we had significant overlap in our sample of females. In further studies, a larger area should be observed to increase the number of females in the study.

In the course of our observations we also noticed that there was no difference in the amount of time spent grooming, calling, or flying by males and females (unpublished data). It did appear, however,

that females in our sample mated more than males (unpublished data), but this was most likely due to the fact that there were fewer females than males in the observation area.

Literature Cited

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Table 1. Summary of differences between male (n = 9) and female (n = 9) individuals of *Jacana*

spinosa in time spent per behavior in a wetland at Palo Verde National Park, Costa Rica.

Behavior	Mean amount of time (in seconds) spent by females in behavior (SD)	Mean amount of time (in seconds) spent by males in behavior (SD)	<i>t</i>	d.f.	<i>P</i>
Foraging*	2104.9 (529.5)	1707.1 (740.8)	1.3	8	0.21
Pecking/Probing	2098.2 (528.2)	1698.3 (737.5)	1.3	8	0.20
Digging	6.7 (8.8)	8.7 (17.1)	-0.3	8	0.80
Territory defense**	42.7 (69.5)	12.9 (11.9)	1.3	8	0.22
Squawking	9.4 (12.4)	4.3 (4.0)	1.2	8	0.26
Displaying	30.4 (71.3)	9.4 (10.0)	0.8	8	0.39
Mediating	2.8 (7.4)	0.0 (0.0)	1.1	8	0.27
Pecking attacks	0.3	0.0	---	---	---
Sitting	0.0 (0.0)	482.8 (660.9)	-2.2	8	0.04

*Foraging was calculated by summing the time spent pecking/probing and digging per bird.

**Territory defense was calculated by summing the time spent squawking, displaying, mediating and in pecking attacks per bird.

The discriminating dragonfly: prey preference in *Leptemis versiculosa* (Libellulidae: Odonata)

Category : Independent Project

Participants : Darren C. Miao

Site : Palo Verde

Key Words : Aposematic coloration, *Catonephele mexicana*, *Eurema dina westwoodi*, *Leptemis versiculosa*, prey preference

Introduction

One of the most abundant inhabitants of Palo Verde National Park, after the blood-sucking mosquito, is *Leptemis versiculosa*, a large, green dragonfly that can be seen flying throughout the day. Yet despite its relative abundance, few studies have been conducted on its feeding and foraging behavior. Observations from a 1988 study (Curey) noted that *Leptemis* discriminate among prey items, specifically avoiding boldly striped insects and baits. Specifically, *Leptemis* did not pursue *Catonephele mexicana*, a small, black butterfly with orange stripes, despite *C. mexicana*'s relatively slow flight pattern. However, the dragonfly was observed chasing and eating other faster and more erratic flying species of the order Lepidoptera such as the brightly yellow *Eurema dina westwoodi* (Curey 1988). It was hypothesized that this discrimination between different butterflies was based on aposematic coloration (Curey 1988). However, Curey admitted that sample sizes in his study were too low to draw definite conclusions.

The purpose of this study is to investigate the hypothesized prey preference of *Leptemis versiculosa*. Two butterflies, the *Catonephele mexicana* and *Eurema dina westwoodi*, are chosen as the differentiating prey items based on Curey's observations of dragonfly

consumption. Two main questions are asked throughout the study : 1) does *Leptemis* exhibit prey preference between the seemingly palatable *Eurema dina westwoodi* and the seemingly aposematically colored *Catonephele mexicana*, and 2) is this selection based on visual cues such as wing coloration or size? It is hypothesized that *Leptemis* exhibits a preference for the *Eurema* butterfly over the *Catonephele* and that the visual cue stimulating this preference is based upon wing coloration and pattern

Methods

This study was conducted Nov. 5 - Nov. 7 at the Palo Verde Research Station in Palo Verde National Park, Guanacaste, Costa Rica. Collections and field tests were performed along the road and airstrip adjacent to the research station. Using an aerial net on the first day, twenty-one *Catonephele* and five *Eurema* butterflies were caught, killed, and pinned open. Body and wing length measurements were made to the nearest tenth of a centimeter.

Four different tests were performed throughout day two and during the morning of day three (see below). A potential prey item (hereafter referred to as a stimulus) was suspended from a 1.65 meter stick with dental floss approximately 1 meter in length. Movement was simulated by swinging the stick slowly while walking

through the test area. The stimuli were carried throughout the test area with one trial being defined as any *Leptemis* response to the stimuli. Stimuli were presented to flying and perched *Leptemis* with the stimuli being swung in front of the test individual at least six and up to ten times before a response was recorded.

Three different types of responses were measured during each test: “no response”, “inspection”, and “hit”. A “no response” was recorded when the *Leptemis* being tested ignored or did not approach the stimuli. An “inspection” was recorded when the dragonfly being tested flew up to the stimuli and briefly hovered in front of the presented stimuli without biting it. A “hit” was recorded when the *Leptemis* took the stimuli into its mouth and attempted to consume it.

Test 1 - Prey preference test: To test for *Leptemis* prey preference, winged *Eurema* and *Catonephele* were presented separately as stimuli. Twenty-three trials were conducted with the winged *Eurema* stimuli, twenty-one trials with the winged *Catonephele* stimuli. Wild dragonflies were used as random test subjects with the same butterfly repeatedly being used as stimuli until it was severely damaged by dragonfly hits. All the trials with *Catonephele* stimuli were conducted first before the trials with *Eurema* stimuli were conducted. The same procedure was employed with all the other tests.

Test 2 - Wing Pattern/Coloration test A: To test the importance of wing coloration on *Leptemis* prey preference, wings of both *Eurema* and *Catonephele* were removed with scissors and the bodies used as stimuli. Twenty-two trials were conducted with the wingless *Eurema*

stimuli, twenty-five trials with the wingless *Catonephele* stimuli.

Test 3 - Wing Pattern/Coloration test B:

To further test the importance of wings as cues in discrimination, wings of both *Eurema* and *Catonephele* were again removed using scissors. *Eurema* wings were then attached onto de-winged *Catonephele* bodies using white glue and dried under a lamp. Likewise, *Catonephele* wings were attached to *Eurema* bodies using the same technique. Twenty trials were conducted with altered *Eurema* stimuli, twenty-one trials with altered *Catonephele* stimuli.

Test 4 - Control test: To test whether the glue affected *Leptemis* prey preference, small amounts of white glue were placed upon the winged bodies of *Eurema* and *Catonephele*. The glue was allowed to dry under a lamp before the glued *Eurema* and glued *Catonephele* were used as stimuli in separate tests. Ten trials were conducted with the glued *Eurema*, fourteen trials were conducted with the glued *Catonephele*.

Once the tests were completed, Wilcoxon non-parametric tests were run to test for significant differences between mean *Eurema* and *Catonephele* body and wing lengths. To determine if significant differences existed between *Leptemis* responses to *Eurema* and *Catonephele*, a chi-squared analysis was performed on the results of test 1. To test for significant differences in *Leptemis* responses to different wing patterns/coloration, eight separate chi-squared analyses were performed on the data collected from tests 1 through 3. “No response” values were excluded from these nine analyses due to the ambiguous nature of the category. A “no response” could be the result of a

dragonfly being full, being lazy etc. instead of it ignoring the stimuli. A separate chi-squared analysis was performed to test for significant differences between the frequencies of “no response” and “hit plus inspection” in tests 1 through 3. In addition, two chi-squared analyses were performed using data collected from test 4 to determine whether a significant difference existed between *Lepthemis* responses to butterflies with glue and to butterflies without. “No response” values were included in the tests because sample sizes were judged too low to allow exclusion from the tests ($n < 15$).

Results

The *Eurema* butterflies used in this study had significantly greater body and wing lengths than the *Catonephele* butterflies (Table 1). However, only 5 *Eurema* were measured in comparison with 15 *Catonephele* due to a low catch count of the former during collection.

Lepthemis “hit” responses to winged *Eurema* were significantly higher than “hit” responses to winged *Catonephele*. Conversely, “inspection” responses to *Eurema* were significantly lower than “inspection” responses to winged *Catonephele* (Table 2). Additionally, *Eurema* “hits” were noticeably stronger and lasted for a longer duration of time (pers. obv.). Dragonflies usually released the *Catonephele* once they had bitten the stimulus (pers. obv.).

No significant difference was observed between *Lepthemis* responses to wingless *Eurema* and *Catonephele* (Table 3). However, responses differed significantly between winged and wingless *Eurema* (Table 4) as well as winged and wingless *Catonephele* (Table 5).

A significant difference was observed in *Lepthemis* response between normal and “altered” *Eurema* (Table 6), normal and “altered” *Catonephele* (Table 7), and “altered” *Eurema* and “altered” *Catonephele* (Table 8). Additionally, “hits” on “altered” *Catonephele* were generally very strong (pers. obv.). In two cases, the “altered” *Catonephele* were carried away from by the dragonfly.

No significant difference was observed between *Lepthemis* responses to normal *Eurema* and *Eurema* with glue (Table 9) nor between normal *Catonephele* and *Catonephele* with glue (Table 10).

No significant difference was exhibited between *Lepthemis* response between normal *Eurema* and altered *Catonephele* (Table 11) or between normal *Catonephele* and altered *Eurema* (Table 12).

No significant difference existed between the frequencies of “no responses” and “hit + inspection” among tests 1 - 3 (Table 13). Percentages of “no response” ranged from 29% to 44% among the three tests.

Discussion

Data from this study indicate that *Lepthemis versiculosa* exhibits preference for *Eurema dina westwoodi* over *Catonephele mexicana* and that this preference is based upon visual cues related to differences in wing pattern and color. Results additionally indicate that the presence of white glue on the butterflies does not affect the preference selection of *Lepthemis*.

Evidence of *Lepthemis* prey preference is based upon the observation that *Eurema* butterflies received a higher

number and greater intensity of hits over *Catonephele*. Evidence that this preference is based upon wing coloration/pattern comes from the observation that this preference could be eliminated through wing removal. Without the wings present, *Lepthemis* apparently cannot distinguish between the two species of butterflies, as evidenced by the similar response pattern to both types of bodies. Additionally, *Eurema* butterflies were not recognized as such by *Lepthemis* when *Eurema* were “disguised” by *Catonephele* wings, and likewise with the *Catonephele* with *Eurema* wings. Instead, response patterns indicate that *Lepthemis* treated altered *Eurema* as normal *Catonephele* and altered *Catonephele* as regular *Eurema*. These results strongly indicate that *Lepthemis* use wing coloration and pattern as their primary basis for prey choice.

Data collected in this study may also support Curey’s (1988) hypothesis that the striped pattern on *Catonephele mexicana* represents aposematic coloration, as all stimuli with *Catonephele* wing pattern and coloration were constantly hit less while being inspected more frequently by *Lepthemis versiculosa*. However, this observation may be the result of body size instead of wing pattern and coloration (see below).

One main issue remains unaddressed by this study and that is the significant differences that occur between *Eurema* and *Catonephele* wing and body size. Because these two factors were not controlled in this study, it is possible that these traits are the cues involved in *Lepthemis* prey preference instead of wing pattern or coloration. *Lepthemis* may prefer *Eurema* over *Catonephele* because

energy reward from the larger butterfly would be higher. However, results from test 2 provide evidence against the body size alternate hypothesis and observations on the natural behavior of the two butterflies provide some evidence against the wing size hypothesis.

Although the *Eurema* body is significantly larger in size, *Lepthemis* did not exhibit a preference for *Eurema* bodies over *Catonephele* bodies, indicating that the dragonfly did not distinguish between body sizes. In fact, “hit” responses on *Catonephele* bodies were twice the number of “hit” responses on *Eurema* bodies, further discrediting the body size alternate hypothesis. Additionally, the highly erratic and fast flight of *Eurema* (pers. obv.) casts doubt upon whether or not *Lepthemis* would obtain a higher net gain of energy reward from the larger butterfly. Capturing *Eurema* butterflies with an aerial net was significantly harder than capturing *Catonephele* butterflies (pers. comm.). It seems likely that *Lepthemis* would also experience such difficulty based upon this anecdotal evidence. Although wing size would make *Eurema* appear larger to *Lepthemis*, the dragonfly would have to expend more energy in pursuing the *Eurema*. Thus, the wing size hypothesis can be called into question, although future studies should be conducted on the question of wing size and *Lepthemis* prey preference.

This study reveals an interesting part of *Lepthemis* behavior and also raises some interesting questions for further study. For example, does *Lepthemis versiculosa* exhibit prey preference with all types of insects, or only within the order Lepidoptera? Do other physical characteristics influence *Lepthemis* prey

preference? Is *Catonephele mexicana* truly unpalatable and does its wing pattern and coloration deter other possible predators? More investigation is needed to answer these questions.

Literature Cited

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Table 1. Wing and body length averages of sampled *Eurema* and *Catonephele* butterflies

	<i>Eurema</i>	<i>Caton.</i>	Z - Value	P - Value	S
Avg. Body Length (cm)					
± 1 Standard Deviation	2.16 ± 0.152	1.58 ± 0.045	2.517	0.0118	5
Avg. Wing Length (cm)					
± 1 Standard Deviation	1.58 ± 0.137	1.11 ± 0.113	3.317	0.009	90

Table 2. Frequency of *Lepthemis* responses to winged *Eurema* and *Catonephele*

	Winged <i>Eurema</i>	Winged <i>Catonephele</i>		
Hit	10	4	$X^2 = 7.036^*$	* - "No response"
Inspection	3	11	$P\text{-value} = .008^*$	values not
			$DF = 1^*$	included in
No Response	10	6		calculations

Table 3. Frequency of *Lepthemis* responses to wingless *Eurema* and *Catonephele*

	Wingless <i>Eurema</i>	Wingless <i>Catonephele</i>		
Hit	5	10	$X^2 = 1.660^*$	* - "No response"
Inspection	8	6	$P\text{-value} = .1976^*$	values not
			$DF = 1^*$	included in
No Response	9	9		calculations

Table 4. Frequency of *Lepthemis* responses to winged and wingless *Eurema*

	Winged <i>Eurema</i>	Wingless <i>Eurema</i>		
Hit	10	5	$X^2 = 3.939^*$	* - "No response"
Inspection	3	8	$P\text{-value} = .0472^*$	values not
			$DF = 1^*$	included in
No Response	10	8		calculations

Table 5. Frequency of *Lepthemis* responses to winged and wingless *Catonephele*

	Winged <i>Catonephele</i>	Wingless <i>Catonephele</i>		
Hit	4	10	$X^2 = 4.014^*$	* - "No response"
Inspection	11	6	$P\text{-value} = .0451^*$ $DF = 1^*$	values not included in calculations
No Response	9	9		

Table 6. Frequency of *Lepthemis* responses to winged *Eurema* and *Eurema* body with *Catonephele* wings glued on

	Winged <i>Eurema</i>	Altered <i>Eurema</i>		
Hit	10	4	$X^2 = 6.312^*$	* - "No response"
Inspection	3	10	$P\text{-value} = .012^*$ $DF = 1^*$	values not included in calculations
No Response	10	8		

Table 7. Frequency of *Lepthemis* responses to winged *Catonephele* and *Catonephele* body with *Eurema* wings glued on.

	Winged <i>Catonephele</i>	Altered <i>Catonephele</i>		
Hit	4	11	$X^2 = 7.813^*$	* - "No response"
Inspection	11	3	$P\text{-value} = .0052^*$ $DF = 1^*$	values not included in calculations
No Response	6	7		

Table 8. Frequency of *Lepthemis* responses to *Eurema* body with *Catonephele* wings glued on body and *Catonephele* body with *Eurema* wings glued on

	Altered <i>Eurema</i>	Altered <i>Catonephele</i>		
Hit	4	11	$X^2 = 7.036^*$	* - "No response"
Inspection	10	3	$P\text{-value} = .008^*$ $DF = 1^*$	values not included in calculations
No Response	8	7		

Table 9. Frequency of *Lepthemis* responses to winged *Eurema* and winged *Eurema* with glue on body

	Winged <i>Eurema</i>	<i>Eurema</i> w/ glue	
Hit	10	5	$X^2 = .138$
Inspection	3	1	$P\text{-value} = .9332$
			DF = 1
No Response	10	4	

Table 10. Frequency of *Lepthemis* responses to winged *Catonephele* and winged *Catonephele* with glue on body

	Winged <i>Catonephele</i> .	<i>Catonephele</i> w/ glue	
Hit	4	4	$X^2 = 1.066$
Inspection	11	8	$P\text{-value} = .587$
			DF = 1
No Response	6	2	

Table 11. Frequency of *Lepthemis* responses to winged *Eurema* and *Catonephele* body with *Eurema* wings glued on

	Winged <i>Eurema</i>	Altered <i>Catonephele</i>		
Hit	10	11	$X^2 = .011^*$	* - "No response"
Inspection	3	3	$P\text{-value} = .918^*$	values not
			DF = 1*	included in
No Response	10	7		calculations

Table 12. Frequency of *Lepthemis* responses to winged *Catonephele* and *Eurema* body with *Catonephele* wings glued on

	Winged <i>Catonephele</i>	Altered <i>Eurema</i>		
Hit	4	4	$X^2 = .013^*$	* - "No response"
Inspection	11	10	$P\text{-value} = .9087^*$	values not
			DF = 1*	included in
No Response	6	8		calculations

Table 13 "No response" and "hit + inspection" frequencies of *Lepthemis* responses for tests 1-3. (*Caton.* = *Catonephele*)

	Test 1		Test 2		Test 3		$X^2 = 2.564$ $P\text{-value} > .05$ $DF = 5$
	<i>Eurema</i>	<i>Caton.</i>	<i>Eurema</i>	<i>Caton.</i>	<i>Eurema</i>	<i>Caton.</i>	
No resp.	10	6	9	9	8	7	
Hit + Insp.	13	15	13	16	12	14	

The breakdown of tristily in *Eichhornia crassipes*

Category: Independent Project
Participants: Sophia Kuo and Brita Dempsey
Site: Palo Verde
Keywords: *Eichhornia crassipes*, self-compatibility, tristily

Haiku: neck deep in the marsh
 mommy told me to stay home
 stinging bugs bite me

Introduction

Many plants are hermaphroditic, having both male and female parts. To prevent self-fertilization and inbreeding, many plants have evolved outbreeding mechanisms. Tristily is a mating system in which inbreeding is prevented by strong incompatibility reactions between sex organs positioned at different heights. In a tristylous flower, two whorls of anthers and one whorl of stigma occur at three different heights: short, mid-length, and long (Charlesworth, 1979). There are three tristylous forms, each named after the position of the stigmatic whorl within the flower. For example, in the short-styled form, the stigma is positioned below one mid-length (*m*) and one long (*l*) anther whorl. The stigma in the mid-styled form is positioned between a short (*s*) and a long anther whorl. In the long form, the stigma is positioned above both the *s* and *m* anthers. Style length of mid-length forms vary greatly. An extreme case in which the stigma is located adjacent to the *l* anther occasionally occurs. This is referred to as semi-homostyly (Barrett, 1983). The three floral morphs often occur in equal proportions (Barrett, 1983).

Each tristylous flower can only be pollinated by pollen coming from an anther

of the same height (Charlesworth, 1979). Therefore, a short morph would need to receive pollen from the *s* anther of another plant. In normal tristylous plants, inbreeding is prevented by strong incompatibility reactions between pollen and stigma from differing whorl heights. Trimorphy in pollen sizes from different anther heights contribute to the incompatibility between sex organs of the same whorl height.

Eichhornia crassipes is a tristylous plant from the lowland tropics of South America. A lack of suitable places for seed germination and inefficient pollination promotes extensive vegetative reproduction, which maintains trimorphy in introduced populations of the species (Barrett, 1979). In sexually reproducing populations, weak incompatibility reactions and very little pollen trimorphism make *E. crassipes* highly self-compatible. Additionally, mid-style forms have a morphology that strongly promotes self-fertilization. Discrepancies in selfing rates between different trimorphic forms favor the creation of monomorphic mid-style form populations (Barrett, 1979).

In the Guanacaste region of Costa Rica, a pronounced dry season lowers wetland water levels, destroying populations of reproducing plants through

desiccation. This reduction in water depth provides *E. crassipes* seeds with an opportunity to germinate, allowing a sexually reproducing population of the plant to persist. In 1979, Spencer Barrett documented the breakdown of the tristylous system in the population of *Eichhornia crassipes* at Palo Verde Research Station in Costa Rica. In contrast to the expected equal numbers of short, mid-length, and long forms, Barrett found the composition of tristylous forms to be 80.2% mid-length forms and 19.8% long stylous forms. No short length forms were seen. Four percent of the mid-style plants he examined were semi-homostylous. The *E. crassipes* at Palo Verde appeared to be evolving from a tristylous system towards a monomorphic population of mid-length forms, possibly in response to poor pollinator efficiency in the area.

If the population of *Eichhornia crassipes* at Palo Verde was evolving away from textbook tristylous in response to inefficient pollination, the system should continue to lose its long-style forms and recruit more mid-style forms. We hypothesized that the percentage of mid-style forms in the population would be higher in 1998 than it was in 1975, at the time of Barrett's sampling. Additionally, if the plants were moving toward an autogamous population, the morphology of the flowers should have changed to reflect the transition from cross pollination to increased self-pollination. Distances between stigma and anthers should have decreased in order to promote pollen exchange within flowers. We hypothesized that during the 23 years since Barrett's sampling, the anther-stigma distance had decreased, and the frequency of semi-homostylous plants had increased.

Methods

The experiment was performed at Palo Verde Research Station in Costa Rica on November 5th and 6th, 1998.

One flower was taken from each accessible inflorescence of *Eichhornia crassipes* in a 100 m² area surrounding the bird tower. The tristylous form of the flower, either mid-style or long-style, was recorded. The distance (in mm) between the stigma and the closest anther was measured using calipers. The following day, the composition of tristylous forms in a 10m² strip adjacent to the airstrip at Palo Verde was recorded. Semi-homostylous flowers were defined as flowers in which the distance between the mid-length stigma and closest long anther was equal to or less than 0.5mm.

χ^2 tests were run to determine whether the composition of morphological types in the 1998 population differed significantly from those from 1979. Specifically, the frequencies of mid-style and long-style forms in the two experiments were compared, using percent values from Barrett's 1979 experiment to calculate the expected values for our study. A χ^2 test was also run to compare the frequencies of semi-homostylous plants in the two samples.

Results

Of our sample size of 242 flowers, 215 (88.8 %) were mid-style forms and 27 (11.2 %) were long-style. No short forms were found. A comparison of our values with that of Spencer Barrett's 1975 experiment (n = 1272, 1020 mid-style forms and 252 long-style forms) indicated a significant difference between the two populations ($\chi^2 = 11.16$, $df = 1$, $P < 0.001$).

The average distance between the stigma and the nearest *l* anther for mid-style and long-style forms were 2.83 mm and 8.44 mm, respectively. In comparison, Barrett's values, 4.5 mm and 10.4 mm, for mid-style and long-style respectively, were 1.7 mm (37.8 %) and 2.0 mm (19.2 %) higher. Because Barrett's data set is not available, we were unable to perform a more rigorous statistical analysis. The magnitude of the percent differences suggest that the values are significant.

Of the 139 mid-style plants whose stigma-anther distance we measured, 7 (5.03 %) were semi-homostylous, in comparison to Barrett's 41 of 1020 (4 %). A comparison of our stigma-anther distance values with that of Barrett's 1975 experiment indicated no significant difference between the two populations ($X^2 = .0389$, $df = 1$, $P > 0.1$).

Discussion

Spencer Barrett was correct when he hypothesized that the population of *Eichhornia crassipes* at Palo Verde Research Station was making the transition from an outcrossing to an inbreeding population. Our data showed that tristyliness in the *Eichhornia crassipes* population of Palo Verde Research Station is continuing to break down in the direction of an autogamous population. This is not surprising. In situations where efficient pollinators are lacking, selection favors individuals that have the ability to self-fertilize. The transition from outcrossing to inbreeding of the Palo Verde population is an example of evolution in action; *Eichhornia crassipes* is responding to selection pressures to increase pollination by taking advantage of a strong self-compatibility. It is possible that in response

to poor pollinator service, the population is moving towards complete autogamy. In this case, over a great period of time, all plants would become semi-homostylous. This scenario would be an extreme case.

The shift toward a higher concentration of mid-style morphs in the population is most likely due to advantages related to the morph's floral morphology and physiology. Mid-style forms are especially well adapted for autogamous reproduction. *Eichhornia crassipes* flowers self when they wither. As the flower wilts, the *l* anthers are pushed into the stigma. Long-style forms are precluded from selfing because their stigmas are located well above their anthers. As the flower withers, the *l* anthers are pushed down away from the stigma. The mid-form is better suited to self; since the *l* anthers are positioned directly above the stigma and the likelihood that they will drop into the stigma is high (Barrett, 1979). Semi-homostylous plants should have the highest selfing rates of all. For selfing flowers, the positioning of anthers and stigma is a strong indicator of reproductive success. Barrett (1979) showed that flowers receiving the most pollen grains were mid-style forms with a very small distance (0-4 mm) separating anthers and stigma. Mid-style flowers whose distance between sex organs was intermediate (4-8 mm) received fewer pollen grains, but still received more pollen than long-styled plants. Mid-style forms also have a strong ability to produce autogamous seed sets. Hand-pollinated autogamy in mid-forms having stigma-anther distances between 0-4 mm produced 66.9 % capsule set (Barrett, 1979). In comparison, long style produced only 0.5 % capsule set. Mid-style forms consistently had a seed productivity than

long-style forms (Barrett, 1979) The average seed set per flower with a stigma-anther distance of 0-4 mm was 48.5 %, compared with 4.0 % for flowers with long style forms. Even in sexually reproducing populations, mid-style forms are at an advantage. Insects collecting pollen are more likely to pollinate the stigma if it is located close to the anthers (Barrett, 1979).

Our comparison of the frequency of semi-homostyly of the 1975 and 1998 populations may not demonstrate a high level of accuracy. We considered any flower with an anther-stigma separation of less than 0.5 mm to be semi-homostylous. The literature is not clear as to how semi-homostyly was defined by Barrett. If Barrett defines semi-homostylous flowers as one in which there is no distance between the stigma and the nearest l anther, then the frequencies of semi-homostylous plants in the 1998 sample are too high. In this case, the frequency of semi-homostylous plants would have dropped between 1979 and 1998. If Barrett considered semi-homostylous plants to be flowers in which the distance between the stigma and nearest anther is less than or equal to 2 mm, than 36.7 % of our population is semi-homostylous, and our reported frequency of semi-homostylous plants is far too low.

To better represent the population of *Eichhornia crassipes* at Palo Verde, future experiments should sample flowers from a larger area. Our sample populations were located close together in a small corner of the swamp, making our values less representative of the population as a whole. Additionally, *Eichhornia crassipes* can reproduce asexually, making it likely that many of the individuals we sampled were clones. This could affect the accuracy

of our data if several of the flows we sampled came from the same genetic individual. Additionally, it might also be revealing to compare the pollen load on the different morphs to examine pollination rates for each form.

In summary, in 1979, Spencer Barrett documented the breakdown of tristily in the population of *Eichhornia crassipes* at Palo Verde Biological Station in Guanacaste, Costa Rica. Barrett found 80.2 % of the plants to be mid-stylous; the remaining 19.2 % of the population showed the long-style morphology. Mean distances between stigma and nearest anther for mid-style and long-style morphs were determined to be 4.5mm and 10.4mm, respectively. We duplicated parts of Barrett's experiment on a small scale to determine whether the breakdown of tristily in the population had continued. A sample size of 242 flowers yielded 88.8 % mid-style forms, and 11.2 % long styled forms. A comparison between mid-style and long style frequencies between the 1979 and 1998 samples showed that the two populations were significantly different ($X^2 = 11.46$, $df = 1$, $P < 0.001$). The mean stigma-anther distances for mid-style and long-style forms were 1.7 mm and 2.0 mm, 37.8 % and 19.2 % lower than in 1979, respectively. Semi-homostylous plants increased in frequency from 4.0 % to 5.03 %, from 1979 to 1998. The population of *Eichhornia crassipes* at Palo Verde is progressing from a outbreeding, dimorphic population toward an inbreeding, monomorphic population of mid-length forms. Selection for autogamy is most likely due to inefficient pollination in introduced populations.

Acknowledgements I'd like to express my deepest gratitude towards Brita Dempsey for being an absolutely wonderful partner and a kick ass woman. Wahoo Brita! I'd also like to thank my Mommy for loving me and feeding me carrot juice. A fondest appreciation is extended to Darren Miao for his admirable efforts in proofreading my paper. Warmest regards also to the courageous Dr. Chris Ivey for his invaluable assistance and the use of his umbrella to "keep it civilized." This work was supported by substantial financial assistance from my padres and by the nice folks at the Williams College Financial Aid Office. Lastly, we'd like to thank the letters R and Q and the numbers 7, 4, and 2.

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Leaf herbivory on *Acacia collinsii*

Category : Independent Project I
Participants: Andrew Knoll, Kristen Ford, and Lisa Stano
Site: Palo Verde
Key words: Ant-acacia, *Acacia collinsii*, herbivory, *Pseudomyrmex*

Introduction

Mutualism between plants and animals is an intriguing example of coevolution. One of the best studied examples of this type of mutualism is the relationship between several species of ants and their host, the acacia tree. An acacia of height greater than 1 m is seldom found without at least one ant species residing in the sharp, hollow thorns that cover the tree's trunk and branches. The tree provides the ants with an excellent source of nourishment with its nectaries and Beltian bodies, and in return the ants defend these food sources and in the process protect the acacia from arthropod herbivores. This relationship enables the tree to reduce its costly loss of leaf biomass to herbivory.

Four species of acacia-colonizing ants are common in Costa Rica, *Pseudomyrmex ferruginea* (rust-colored), *P. belti* (large black-colored), *P. nigrocinta* (yellow-colored), and *P. crematogaster* (small, black-colored). The *Pseudomyrmex* ants defend the tree against herbivores, but the *Crematogaster* ants are not involved in active defense (Janzen 1983). Although an acacia is well-defended by its symbiotic ants, this is not to say that the tree is free from herbivory. In many cases, acacias with resident ants will still suffer heavy losses to herbivores. Also, the amount of leaf herbivory may vary widely among individuals in the same area.

These observations lead to some obvious questions: What factors could account for this wide variation? Are some ant species superior to others as defenders of acacias? Are leaves of a certain age defended more vigorously than others? Does leaf herbivory, or defense against it, vary with habitat? These are the questions which we undertook to investigate with this study. Based upon Janzen's descriptions of the ant species, we hypothesized that leaf herbivory would vary with colonizing ant species. We also predicted that older leaves would suffer heavier herbivory losses than younger leaves due to loss of Beltian bodies as leaves age, and that the herbivory level of acacias would vary significantly between habitats due to varying species diversity and total production in different forests.

Methods

Our study was conducted at OTS's Palo Verde Biological Reserve in Palo Verde National Park in the Guanacaste province of Costa Rica. We chose the species *Acacia collinsii* because it is the most abundant of the acacia species found in Palo Verde. This species favors light gaps and relatively wet soils. We initially visited a primary forest and did not observe any acacia species in this area. Therefore, we chose to use secondary forest as our habitat of study, and selected 3 representative type of secondary forest :

abandoned pasture (3-4 years since abandonment), 8-10 year-old secondary forest on both sides of the access road, and 30 year-old secondary forest behind the Palo Verde campground. On November 4 and 5, we visited each of the 3 sites and observed 40 trees at each site. On each individual tree we recorded percent herbivory data for 10 young leaves and 10 older leaves. Younger leaves are easy to distinguish because of their characteristic lighter green color and their usual location at the ends of branches. We estimated the percentage of leaflets lost or destroyed on a leaf compared to the approximate total number of leaflets that would be present on an undamaged leaf. In this way, we were able to classify leaves according to specific ranges of herbivory : 0, 0-10 %, 10-25 %, 25-50 %, 50-75 %, and 75-100 %. In addition, we recorded the species of ant which was present on each tree and took note of whether there were other *collinsi* in close proximity.

We used a 2-function ANOVA test to see whether a significant difference in leaf herbivory existed between the young and old leaves we sampled. This was done to account for the fact that we used paired samples of young and old leaves from each tree rather than sampling all our leaves at random. We also used a 2-function ANOVA test to see whether the species of ant found on the tree significantly affected the level of leaf herbivory. The Tukey-Kramer Test of Multiple Comparisons was used to compare the 3 habitats simultaneously with respect to leaf herbivory.

Results

Our results showed that a significant difference existed between the leaf

herbivory of the young and old leaves we sampled, both overall (F -ratio = 136.50) and within each habitat (F -ratio = 35.04 for abandoned pasture, F -ratio = 88.03 for early secondary forest, and F -ratio = 20.82 for older secondary forest.) The 2-way ANOVA test produced a P -value of $P < 0.0001$. The mean leaf herbivory percentages for each habitat are indicated in Table 1.

The Tukey-Kramer Test of Multiple Comparisons showed that a significant difference in leaf herbivory existed between the early secondary and older secondary forests ($q = 7.188$, $q^* = 2.35$, $P < 0.05$) and also between the older secondary forest and the abandoned pasture that we investigated ($q = 5.669$, $q^* = 2.35$, $P < 0.05$). However, our data showed that there was no significant difference in leaf herbivory between the abandoned pasture and early secondary forest ($q = 0.756$, $q^* = 2.35$, $P > 0.05$).

All four potential ant species were observed protecting acacias . The 2-function ANOVA test revealed that the ant species present on the tree did not significantly affect the tree's leaf herbivory percentage ($P = 0.5763$). Excluding data for species observed less than 3 times and for trees with multiple species did not alter this finding ($P = 0.5755$).

Discussion

Our results confirmed our prediction that leaf herbivory is significantly higher for older acacia leaves than for younger leaves. There are several possible explanations for this trend. Older leaves have been exposed to herbivores longer than younger leaves. If herbivores continue to feed on leaves as they age then older leaves will have experienced more

herbivory than younger leaves at a given time. Younger leaves may also be more vigorously defended by ants because young leaves produce more nectar than old leaves (Dodd, this volume). Similarly, ants may preferentially defend young leaves because Beltian bodies, which are an important protein source for ants, are produced at the tips of leaflets when the leaves are in very early development (Torres, et al 1977). Thus, older leaves, which have lost a substantial number of Beltian bodies, may be less attractive to ants. A final hypothesis, for which we have no concrete evidence, is that younger leaves may be more distasteful or less nutritious than older leaves, thus decreasing their desirability to herbivores.

The results of the Tukey-Kramer Test revealed that overall, there were significant differences in leaf herbivory between the three habitat types we investigated. Early successional forest showed the most leaf herbivory, while abandoned pasture showed less herbivory but not to a significant degree and older secondary forest had significantly less herbivory than the other two habitats. The lower biomass and total productivity of the shrubbery in the older secondary forest may be the cause of this trend. Damage by generalist herbivores may be directly proportional to the overall biomass of productivity of an area. In other words, a denser, richer food source tends to attract greater numbers of generalist herbivores. We observed that shrubbery in the older secondary forest was much less dense and the shrubs were generally not as massive as the other two habitats, enhancing the likeliness of this explanation. Another hypothesis is that increased species diversity may lead to increased generalist

herbivory as well (Mitchell, et al 1997). It may be for this reason that the older secondary forest, where species diversity seems to be lower, is less susceptible to herbivore attacks. Future experiments should sample species diversity, biomass, and productivity of the sampled areas to test this hypothesis.

Our ANOVA results indicated that the ant species present on an *acacia* did not significantly affect the level of herbivory. Our data leads us to conclude that at Palo Verde, the four ant species we observed were roughly equal in their ability to defend acacias against herbivory. We hypothesized that this trend may be due to the fact that ants defend primarily nectaries and Beltian bodies more so than leaves. We therefore may not have seen a true difference in ant protective ability that existed because we only sampled leaves. Experiments in the future should perhaps take data on nectary and Beltian body herbivory rather than only leaf herbivory.

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Table 1 : Percent herbivory means for young and old leaves across three habitats

Leaf Age	Abandoned Pasture	Early Secondary Forest	Older Secondary Forest	Overall
Old	23.63%	27.57%	13.81%	22.00%
Young	15.54%	14.69%	9.41%	13.37%
Overall	19.61%	21.13%	11.59%	-----

Differences in perching behavior among four morphospecies of Anisoptera

Category: Independent Project
Participants: Michelle Hersh, Susannah Nicholson, and Cory Tyzka
Site: Palo Verde
Key words: Anisoptera, dragonfly, ecological niche, Odonata, perching behavior

Introduction

At least five different dragonfly morphospecies (Order Odonata, Suborder Anisoptera) coexist in the marshside abandoned airstrip habitat of Palo Verde National Park, Guanacaste, Costa Rica. Most of these morphospecies potentially share prey and foraging areas, oviposition sites, and other resources. Somehow, the available resources must be partitioned among the many individuals in the Anisoptera community, by direct competition or other means. One behavior that may be indicative of this resource division is perching habits; the purpose of this study was to determine if perching behavior differed among four of Palo Verde's Anisoptera morphospecies.

Anisoptera may perch for many reasons, including foraging more efficiently for prey, guarding a foraging or mating territory, waiting for potential mates, or hiding from predators. To accommodate these potentially different types of behaviors, we predicted that dragonflies may alter the time they perched, perch preferentially at certain altitudes, habitat types, or perch types, visit certain perches more frequently than others, or distribute their time spent perching and flying in a particular way. Carey (1988) observed altitudinal division of foraging habitats among three morphospecies (yellow, green and an additional red morphospecies) at the same location. By comparing these general

behaviors, we wanted to begin to understand if these morphospecies occupied different realized ecological niches.

Methods

Dragonflies are highly opportunistic, voracious predators of other insects; they capture their prey on the wing using their legs as a basketlike sieve (Hogue 1993). Common prey types include Diptera, Coleoptera, Hemiptera, Lepidoptera, and in some cases, other Odonata. Dragonflies hunt singly or in aggregations, and reside in habitats near marshes, streams, lakes or other standing water (Hogue 1993). Most females oviposit directly onto standing water, and nymphs are entirely aquatic. Among adults, habits of flight, including height and speed, are often characteristic for species (Borror et. al. 1989).

This study took place between 0800 and 1600 on November 6, 1998, and 0800 and 1000 on November 7, 1998, on the airstrip between the marsh and the main road of Palo Verde National Park, in Guanacaste, Costa Rica. The total section of the airstrip sampled was approximately 10 square meters. The site was selected because of the abundance of four different Anisoptera morphospecies in the area. The morphospecies are referred to by body color: solid black, light brown with a wide yellow stripe (yellow), dark brown with faint mustard striping (brown) and bright

green. The green morphospecies was identified as *Leptemis versiculosa*, in the Libellulidae family (Miller 1983).

Each morphospecies was observed in ten-minute intervals for the following times (in minutes): Black-71.78, Brown-72.75, Green-72.5, Yellow-70.0. The type of morphospecies observed was alternated among the four throughout the testing period. It was originally intended that each individual would be marked before it was observed, but it was soon realized that the act of marking affected the behavior of the individual and could not be done. The perch time (measured by stopwatch), estimated perch height, location, and type were all recorded each time a perching individual was observed. Additionally, the number of times the individual visited each perch was noted. Perch locations were defined as either *air strip*, the grassy, sunny center of the airstrip, *near marsh*, the short vegetation less than one meter from the marsh, *marsh*, the vegetation directly over the edge of the marsh, and *standing water*, a small flooded area on the opposite side of the air strip. Perch types were classified into the following categories: grass, Malvaceae leaf, leaf of non-Malvaceae small plant, fallen twig, *Acacia* tree branch, non-*Acacia* tree branch, rock, ground, net, and poncho (The last two categories being landings on equipment brought into the field). Malvaceae leaves were placed in a separate category because the family was very abundant and selected frequently as a perch site by the Anisoptera.

The data collected were analyzed using the JMP statistical analysis package (SAS 1989-1997). The mean perch times and mean perch heights by morphospecies were compared using ANOVA, and

significant differences among the means were identified using the Tukey-Kramer test. A chi-square analysis was performed to determine if the distributions of perch location and type were non-random and different among morphospecies. Additionally, the evenness of perch diversity per morphospecies was calculated based on the number of times individuals of each morphospecies visited each different perch. The formula for evenness, which uses the Shannon-Weiner diversity index, is described by Begon et al. (1996). Finally, the total percentage of time each morphospecies spent in flight was calculated by subtracting the total amount of perch time from the total amount of observation time, then dividing that answer by the total amount of observation time.

Results

The hypothesis that differences in perching behavior existed among morphospecies was generally supported by the data collected. The mean perch times were statistically different among all four morphospecies, ($F_{3,228} = 30.182$, $P < 0.0001$) though individual perch times varied greatly around the mean (Table 1). Each of the four means was statistically different from each other mean ($P < 0.05$). There was no difference found between the mean perch heights of the four morphospecies ($F_{3,118} = 0.7068$, $P = 0.5498$). Additionally, a statistical difference was found in the distribution of both perch location ($X^2 = 84.2$, $df = 231$, $P < 0.0001$) and perch type ($X^2 = 110.7$, $df = 229$, $P < 0.0001$). The percentage of perches per morphospecies at each habitat location are summarized in Table 2. The most common perch types and the percentage of perches per morphospecies

on that particular type are as follows: Black: Grass (20 %), Malvaceae leaves (36 %), Other leaves (33 %). Brown: Grass (45 %). Green: Malvaceae leaves, (32 %) Other leaves (21 %). Yellow: Grass (66.7 %), Other leaves (22 %). The evenness of perch diversity was highest in the yellow and lowest in the black morphospecies (Table 1). Green dragonflies spent a far greater percentage of time in flight than the other three morphospecies, each of which spent over 90 % of their time perched (Table 3).

Discussion

The results of this study indicate that there is a significant difference between the perching behaviors of the four morphospecies of Anisoptera observed. Summaries of each morphospecies' behaviors are as follows:

The behavior of the green dragonflies was most distinct compared to that of the other morphospecies. They were the most active of the four morphospecies; they spent over half their time in flight, had the shortest perch times, and tended to use a wide variety of different perches. Miller (1983) also observed that this morphospecies perched less often than other Odonata and encountered prey by chance. Sherman et. al. (1993). observed this morphospecies for 4.95 person-hours and found that it spent 29.4 % of its time in flight, 64.2 % perched, and 6.4 % hovering, chasing prey, or interacting with other individuals. Their most common perch sites were Malvaceae or other small herbaceous plants; they also perched less often than the other morphospecies on grass, possibly because the body weights of some individuals were too large to be supported by it.

Black dragonflies had the second-lowest average perch time, yet spent the majority of their time perched and displayed the lowest amount of perch diversity. The evenness statistic was significantly affected by one individual who was observed jumping on and off the same perch twenty-one times. Some individuals were very sedentary, barely or not moving from the same perch, while others were nearly as active as the green morphospecies. The majority of black dragonflies were found in or near the marsh, perching on grasses or small plants including Malvaceae.

Brown and yellow Anisoptera morphospecies displayed very similar behaviors. Both had relatively long mean perch times, spent the vast majority of their time perched, and utilized diverse perches. They were relatively inactive and camouflaged themselves well in the surrounding area while perched, probably a behavior involved in remaining unseen by predators and potential prey. The yellow morphospecies was concentrated more near the standing water habitat, while the brown was observed by the airstrip or near the marsh area; perhaps these two morphospecies are partitioning resources based on perch location.

The most probable causes of experimental error are pseudoreplication and difficulties in both following fast individuals and estimating perch heights during very brief perches.

A preying order among the morphospecies was observed. Green preys on brown and black, who in turn prey on yellow (pers. obs.). The yellow morphospecies then has more potential predators than the brown and black, which have more than the green. Green individuals spend far more time in motion and thus are

more visible to predators than yellow individuals, who have longer mean perch times and spend little time in the air. A typical behavior of the green morphospecies was hovering in one place rather than perching and flying in large, erratic circles around the same section of airstrip. The other three morphospecies were often seen waiting at one perch site, then jumping quickly and suddenly for insects flying in the general vicinity (pers. obs.). However, this preying order is based only on anecdotal evidence, and is just one of many potential explanations for some aspects of these behavioral differences. Preying order among dragonfly morphospecies could be an interesting future study.

Do the overall behavioral differences possibly imply that the morphospecies inhabit different realized ecological niches? Preliminary results seem to indicate that at least the green morphospecies exists in a different niche than the other three morphospecies because of its distinct foraging style and status as top dragonfly predator, although some differences exist between the other three morphospecies as well. Obviously, much further study in topics such as prey choice, flying and perching heights, mating and oviposition sites, territoriality and competition, and other factors is necessary before something as complex as an ecological niche can be defined.

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Table 1: Mean time on each perch and perch diversity statistics for four morphospecies of Anisoptera in Palo Verde National Park, Guanacaste, Costa Rica.

Morphospecies	# of times perched	Mean perch time (St. Dev.) (sec)	# of different perches	Evenness of Perch Diversity (J)
Black	71	54.9 (120.9)	36	0.31
Brown	20	158.4 (207.5)	14	0.58
Green	123	14.6 (28.8)	69	0.64
Yellow	18	254.5 (229.8)	16	0.94

Table 2: The distribution of locations of perches used among four morphospecies of Anisoptera in Palo Verde National Park, Guanacaste, Costa Rica. Each numerical value represents, for each habitat type, the percentage of the total number of perches each morphospecies made at that particular habitat type.

Morphospecies	Airstrip	Marsh	Near Marsh	Standing Water
Black	16.9	12.7	67.6	2.8
Brown	40	10	45	5
Green	52.5	1.6	44.3	1.6
Yellow	16.2	5.6	11.1	66.7

Table 3 The percentage of time four morphospecies of Anisoptera in Palo Verde National Park, Guanacaste, Costa Rica, spent in flight and perched.

Morphospecies	% time spent in flight	% time spent perched
Black	9.6	90.4
Brown	5.1	94.9
Green	59.3	40.7
Yellow	4.7	95.3

The effect of *Pseudomyrmex* ants on *Acacia* Beltian body consumption

Category: Independent Project
Participants: Carl F. Salk and Raivo-Erik R. Vihman
Site: Palo Verde
Key Words: *Acacia collinsii*, *Acacia cornigera*, beltian bodies, mutualism, *Pseudomyrmex*

Introduction

The relationship between bull's horn acacias (*Acacia collinsii* and *A. cornigera*) and "acacia-ants" (*Pseudomyrmex ferruginea* and *P. belti*) is an archetypal coevolved mutualism. The acacia trees benefit in two ways from the ants' presence. First, the ants attack and repel herbivores. Second, the ants remove competing vegetation from a circular patch around the base of the tree. In turn, the ants receive three benefits from the acacias. Acacias provide protected homes for ants inside large, hollowed-out spines. Acacias also provide food for the ants. Extrafloral nectaries near the base of each petiole secrete a sugar rich solution. The ants also consume growths of protein rich cells found at the tip of each secondary leaflet of the bipinnately compound leaves (Janzen, 1983). These growths, termed "Beltian bodies", are the focus of this study.

Our particular interest in Beltian bodies deals with their rate of consumption. This is indicative of two different things. Beltian body consumption can be the result of either a vigorous ant colony or the presence of Beltian body "predators" who do not benefit the plant in any way. Both of these situations have consequences for the acacias' ability to grow and reproduce. A previous OTS course project quantified the rate of Beltian body disappearance (Blaney, 1989). Our study aims to go one step further and compare the rate of

consumption between acacias which have been colonized by ants and those which remain uncolonized. We hypothesize that Beltian bodies will be removed from plants regardless of the presence of ants, but that they will be removed more rapidly from ant-colonized plants than from uncolonized acacias.

Methods

On November 6, 1998 we identified 35 bull's horn acacias near the airstrip in Palo Verde National Park, Costa Rica. No attempt was made to distinguish between the two acacia species present in the area. We examined each tree to determine whether it had been colonized by ants. In the case of individuals with no obvious ants a branch was shaken vigorously to see if any appeared. Only ants of the genus *Pseudomyrmex* were considered. On each plant we chose one to six leaves based on the presence and visibility of their Beltian bodies. We then counted these Beltian bodies and noted the time. Each leaf was marked for future identification by placing a small flag of masking tape on the twig abaxially from the petiole base. Six to eight hours later we returned to the plants and repeated our census, once again recording the time and number of Beltian bodies present on each leaf.

Using these data we calculated the rate of Beltian body disappearance in two

different ways. The first method yielded the absolute per-hour rate of Beltian body consumption, R_a :

$$R_a = (BB_i - BB_f) / (T_f - T_i),$$

where BB_i and BB_f equal the number of Beltian bodies present in the initial and final censuses, respectively, and T_i and T_f are the time in hours of each census. The second calculation resulted in the percentage of Beltian bodies consumed per hour, R_p :

$$R_p = 100 * (BB_i - BB_f) / [BB_i * (T_f - T_i)].$$

The difference in R_a and R_p between individuals with and without ants and between all individuals was analyzed using two-way ANOVAs performed with the JMP statistical package (SAS Institute, Inc., 1996).

Results

Ants of both species were found on our acacias. Although exact frequencies were not counted, *P. ferruginea* predominated. Neither species of ant showed an exclusive preference for either kind of acacia. Several taxa of insects were observed on the plants. Praying mantises were common on ant-occupied plants while caterpillars, beetles and walking sticks were seen on unoccupied acacias. However, no insects other than *Pseudomyrmex* were observed collecting Beltian bodies. A few small acacia plants were densely covered in climbing vines; these plants were unoccupied.

Both methods of calculation yielded a higher rate of Beltian body consumption for colonized acacias than for uncolonized individuals (Table 1). The mean R_a and R_p values for ant-occupied plants were respectively 4.3 and 3.1 times greater than for unoccupied plants. Both of these differences were statistically significant

(Tables 2,3). The two-way ANOVAs also revealed a significant difference between individual plants, regardless of treatment, for R_a but not R_p (Tables 2,3).

Discussion

Our data imply that most Beltian bodies produced by ant-occupied bull's horn acacias are consumed by *Pseudomyrmex* ants. This is evolutionarily advantageous to the plants as long as they are successfully defended against herbivores and competing plants. Our qualitative observations suggest that the acacias are indeed being defended by *Pseudomyrmex* ants. The only insects commonly found on occupied plants were mantids which are exclusively carnivorous (Hogue, 1993). Similarly, ant-occupied trees were free of climbing vines and other vegetation around the base of their trunk.

The presence of carnivorous mantids on ant-occupied plants is puzzling. If they were to eat vegetable matter, Beltian bodies would be a likely food item since they are mostly protein, as are the mantids' traditional prey items. However, this does not explain why the mantids would be found on occupied rather than unoccupied acacias. First, occupied acacias have the obvious disadvantage of being defended by biting, stinging, ants. Second, competition for Beltian bodies is much less fierce on unoccupied plants. Third, the mantids' usual prey items are more common on uncolonized acacias.

Beltian bodies on unoccupied plants are an undefended, protein-rich food source. As such, they must be particularly attractive to opportunistic herbivores. While only *Pseudomyrmex* ants were observed collecting Beltian bodies it is probable that the insects we observed on

unoccupied plants were responsible for the slow disappearance of Beltian bodies on these plants. In this case, the acacia loses valuable amino acids without gaining the protection of the ants. However, even if unoccupied acacias could produce leaves without Beltian bodies, to do so would not be advantageous. This would eliminate an unoccupied plant's chances of ever attracting a new ant colony. When a queen finds an acacia in which to establish her new colony she immediately collects Beltian bodies and nectar to feed her first clutch of workers (Janzen, 1983). Even if a queen did choose a plant without Beltian bodies the ants' dependence on this missing food source would probably render the colony a failure.

Several factors could have caused this. One is that there are actual differences between plants or their ant colonies that influence local Beltian body consumption rates. Possible differences include protein content of Beltian bodies or population densities of ants. Perhaps these differences are merely a mathematical artifact. Many of the sampled plants contributed only one leaf to our data set, and none contributed more than six. Also, the difference between plants was significant for the absolute difference, but not the percentage difference in Beltian body consumption. This second method of calculating consumption is probably more biologically accurate since absolute consumption rates depend are related to the number of Beltian bodies present at the beginning of the experiment. Whatever the case may be, the effect of ants was still stronger than the inter-plant differences.

Several questions relating to this study need to be addressed in future research. The ecological role of mantids on

ant-occupied acacias needs to be elucidated. The insects responsible for consumption of Beltian bodies on unoccupied plants should be identified. Also needed is a similar study addressing the possibility of nectar consumption on unoccupied plants.

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Table 1. Average rates of Beltian body disappearance from acacias at Palo Verde, Costa Rica with and without ants present. R_a = mean rate of Beltian body consumption per hour. R_p = mean percentage of Beltian bodies consumed per hour, as measured over six to eight hour periods.

plant condition	R_a	R_p
ants present	6.068	9.364
ants absent	1.388	2.993

Table 2. Two way ANOVA results for absolute Beltian body consumption rate from acacias at Palo Verde, Costa Rica. For whole model test $F_{34,22} = 4.5574$, $P = .0002$.

effect	DF	F	P
plant number	33, 22	3.284	.0024
presence of ants	1, 22	16.242	.0006

Table 3. Two way ANOVA results for percentage of Beltian bodies consumed per hour. For whole model test $F_{34,22} = 2.113$, $P = .0344$.

effect	DF	F	P
plant number	33, 22	1.101	.4139
presence of ants	1, 22	5.292	.0313

Alouatta palliata and their forage selectivity of trees

Category: Independent Project
Participants: Jesse Greenston and Abigail Keys
Site: Palo Verde
Keywords: *Alouatta palliata*, folivory, foraging, primates.

Introduction

Foraging is an activity that animals do in order to encounter food sources. It is critical for animals to differentiate among food sources to find the most optimal diet (Janzen 1983). Folivores should avoid feeding on toxic secondary compounds, spines, and other defense mechanisms trees have evolved (Janzen 1983).

Previous studies of Howler monkey foraging behavior have suggested that they are selective feeders. Glander (1975) observed howler monkeys and recorded the age and quantity of leaves the monkeys ate. He found that the monkeys fed selectively on the basis of leaf age. During a one year study, leaves made up 63.6 % of their diet (Glander 1975). New leaves composed 44.2 % of their diet as compared to 19.4 % of old leaves (Glander 1975). Young leaves generally have more water, less fiber, and fewer secondary compounds which makes them better food sources (Gentry 1993).

In this study we investigated whether howler monkeys exhibit selective foraging behavior, feeding from only a small portion of available trees. Our predicted results are that the monkeys will selectively feed from trees that have small concentration or no secondary compounds or other attributes that discourage folivores.

Methods

Howler monkeys inhabit lowland and montane forest (up to 2500m of altitude), and are found from southern Mexico to northern South America.

The study site chosen was a 20 x 50 m area of tropical dry forest at the Palo Verde Biological Station in Guanacaste, Costa Rica. Five howler monkeys were observed on November 6, 1998, from 5:55 am to 9:00 am, for approximately 185 minutes. The observations were recorded by Abigail Keys and myself. Trees that the howlers fed from were recorded as well as the amount of time they spent in each tree. The time recorded in each tree was done on the monkey leading the troop. This was done because the rest of the monkeys eventually followed and there were not enough investigators to make accurate observations on all of the monkeys.

Leaf samples were collected and identified from all trees in a 1000m² area with a Diameter Breast Height (DBH) greater than 40 cm. Maguil Cespedes identified all the tree samples found in the area. This area was selected because it enclosed the trees that the monkeys were observed foraging through during the time of observation. Only trees with a DBH \geq 40cm were included in the study because we estimated that trees with this DBH could support howler monkeys and therefore be a possible food source. Tree characteristics were determined by referencing every species found in our area in Gentry (1993).

A chi-square test was used to compare the amount of time monkeys spent foraging in each tree with the amount of time they were expected to forage in each tree if they foraged randomly. The expected foraging time per tree was determined by calculating the percent trees represented by a species multiplied by the 185 minutes that the monkeys were observed. The resultant value was an estimation of the amount of time monkeys would spend per tree species if they randomly foraged.

Results

The null hypothesis of the statistical test is that the monkeys will randomly feed from the 23 tree individual's found in our transect area. There were 23 tree individuals within 14 species found in the 1000m² area (see Table 1). From the value of the chi-square test, the null hypothesis could not be accepted (chi-square value = 153.51, alpha = .05, p value = 22.36, df = 13). Of the 185 minutes of observation, the monkeys spent a disproportional amount of time in *Piscidia carfuginensis*, approximately 105 minutes.

Discussion

Our hypothesis that howler monkeys selectively forage for leaves was supported by our data. This agrees with data presented by Glander (1975).

Possible reasons for monkeys exhibiting selective behavior largely has to do with the attributes of the trees. Many trees produce secondary compounds which renders them difficult to digest or toxic to the howler monkeys (Gentry 1993). *Meriospermum frutencens*, a tree found in our transect that was not sampled by the monkeys has potent secondary compounds toxic to most mammals. Other trees

produce spines on the leaves and bark to discourage herbivores. *Randia thurberi*, a tree found in our transect produces spines on its leaves and bark and was not fed from (see Table 1). All but one of the trees that were foraged on by the howler monkeys did not have secondary compounds (see Table 1).

Another reason that our hypothesis could have been supported is because our data could have been faulty. There was one large outlier in our data that we chose to include. This was the large amount of time that the howler monkeys spent in *P. carfuginensis*. During our observation period it rained intermittently and the howlers appeared to be more sedentary during the rain. Although, we did not prove this statistically. During the period of heaviest downpour the howlers stayed in *Piscidia*. Therefore, they could have remained in *Piscidia* for such an extensive period of time only for shelter from the rain. If that were the case, it would have skewed the results of our chi-square test dramatically. Because the majority of time observing the howler monkeys took place in *Piscidia*, we chose to include the data.

Future suggestions for the investigation would be a larger time of observation. The investigator should take into account changes in climate to ensure it would not effect the data. Another way to modify the experiment would be not only to include the trees the howler monkeys are foraging from, but also to analyze the leaves from the trees. This would be useful to identify how concentrated the secondary compounds are in the leaves and if that has a significant influence on selectivity, as we have suggested.

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Table 1. Expected vs. Observed Values of Time Howler Monkeys Spent Foraging per Tree Species

Tree Species found in transect	Observed amount of time monkey spent in tree (minutes)	Expected amount of time monkey would spend if randomly foraging (minutes)	Presence of secondary compounds	Number of individuals found in 1000 m ² area
<i>Apeiba tibourbou</i>	0	8.04	yes	1
<i>Brosimum alicastrum</i>	8	8.04	yes	1
<i>Chomelei soinoza</i>	10	16.09	no	2
<i>Forsteronia spicata</i>	13	16.09	no	2
<i>Guacum santum</i>	0	8.04	yes	1
<i>Guazuma ulmifolia</i>	0	32.17	yes	4
<i>Luhea candida</i>	40	40.22	no	5
<i>Merospermum frutencens</i>	0	8.04	yes	1
<i>Piscidia carfuginensis</i>	105	8.04	no	1
<i>Randia thurberi</i>	0	8.05	spines, no	1
<i>Spondis mombin</i>	0	8.04	no	1
<i>Stemmedenia obovata</i>	0	8.04	no	1
<i>Tabeluia ochracea</i>	9	8.04	no	1
Unknown	0	8.04		1

Differences in gender-based territorial aggression in *Jacana spinosa*

Category: Independent Project
Participants: Jonathan P. Micancin
Site: La Selva
Key Words: Aggression, gender differences, *Jacana spinosa*, polyandry, territoriality.

Introduction

Gender-based aggression, or aggression by and between different sexes, is a common subject of study for animal behaviorists and social biologists, since it is often the defining aspect of the social systems of many animal species. Furthermore, understanding gender and aggression in other species has recently become an important tool in understanding human behavior, from playground fights to domestic abuse. One species in which gender-based aggression has been heavily studied is *Jacana spinosa*.

Jacana spinosa, the northern jacana, is a common and highly visible waterbird species of the Neotropics from Panama north through Mexico and Texas (Stiles 1989). Within this range the species populates marshes and other vegetation-covered bodies of water, where its distinctively long toes and feet allow it to walk on the surface of the water as it forages and breeds. *Jacana spinosa* has been studied because of its unusual mating system (simultaneous polyandry), in which one female mates with and lays eggs for several males within the same breeding season (Jenni 1983). Breeding females each establish a territory within which are included the foraging and nesting territories of several males, her "harem." They tenaciously defend this territory and the males contained within it from the advances of other females throughout the breeding

period, through loud vocalizations, displays of the bright yellow underwings, and outright physical attacks. Males are also strongly aggressive toward other males infringing upon their territories (Jenni 1983), and females will aid males in their territory with defending these territories from other males in the harem as well as entirely foreign males (Jenni 1983). Females are as much as 75% larger than males, enhancing their effectiveness as defenders.

Since females defend not only their territories and males but also aid their mates in territorial defense, females might be expected to engage in aggressive/territorial behavior more frequently than males. The goal of this study was to test the hypothesis that female *J. spinosa* exhibit a higher frequency of aggressive/territorial behavior than males.

Methods

The study was conducted on November 5, 6, and 7, 1998 at the Palo Verde Biological Station of the Organization for Tropical Studies in Guanacaste Province, Costa Rica. Observations of *J. spinosa* were made from the bird tower overlooking an area of the marshlands south of the Rio Tempisque.

On the morning of November 5, aggressive/territorial interactions between fourteen *J. spinosa* individuals which consistently remained closest to the bird tower were observed to identify the

territories of females and the number of males within each territory. Females were distinguished from males by their larger size and from one another by their territory locations. Males were consistently associated with particular female territories but could not be distinguished from one another.

On November 6 and 7, six total hours were devoted to observing and recording descriptions of all aggressive/territorial interactions within the female territories. Aggression was defined as any action which caused the displacement of another individual. These included vocal and physical displays as well as charging and pecking. Aggressors and "victims" were identified by territory and their actions noted. Afterward, the number of aggressive/territorial acts committed by each female and all the males within each female territory were totaled from these observations. Aggressive frequencies of females were defined as the total number of aggressive acts committed by an individual female. Mean male aggressive frequency was calculated by dividing the total number of aggressive acts by the number of males for each territory. Overall mean aggressive frequency was determined for each gender by dividing total aggressive acts by the number of individuals of each sex. These results were then statistically analyzed using χ^2 tests.

Results

Three female territories were identified. Territory 1 contained one female and five males, while Territories 2 and 3 each contained one female and three males. Thirty-two aggressive episodes were observed (Table 1, 2), during which three types of aggressive behavior were

identified: 1) females acting aggressively toward females; 2) females acting aggressively toward males (of their harems and of the harems of other females) during conflicts with a male in the females' harems; and 3) males acting aggressively toward other males. Ten of the seventeen aggressive acts committed by females were of type 2 (Table 1). Therefore, in ten of the twenty-five aggressive acts by males, the aggressive male was assisted by its mate. This occurred five times in Territory 1, four times in Territory 2, and only once in Territory 3.

Total frequencies of aggression for males and females within gender did not show a significant difference ($\chi^2 = 0.0$, $df = 1$, $P = .05$). Female aggression toward males included, however, yields a significant result ($\chi^2 = 5.02$, $df = 1$, $P = .05$).

Discussion

The data show that no difference exists in the frequencies of aggression of male and female jacanas toward their own genders. Females, however are considered more aggressive if attacks on males are considered along with attacks on females.

An adaptive advantage may exist to explain why females are more aggressive than males. Due to sexual selection, *J. spinosa* females necessarily exceed males in two ways. First, females are more aggressive than males, which provides an advantage during competitions with other females for large territories and mates to fill them. Second, females are larger than males to provide resources for the production of many eggs to fill the nests of several males, aid in competition against females, or both. Larger and more aggressive females appear to be naturally better suited toward territorial behaviors

than are smaller, meeker males. In turn, females are better suited to defending the territories of the males in their harem from intruding males in addition to their own larger territory since their larger size makes them easily capable of dominating any male in an aggressive encounter. Individuals that defend the territories of their males have an adaptive advantage. The males incubates the clutch and locates food for the young of the pair. Thus, males cannot afford to participate in frequent and potentially dangerous territorial conflicts with other males. If a male were to be injured or die, the young would likely suffer as well. Males aided by their mates in defending a territory are therefore much more likely to raise the young to maturity, increasing the frequency of “big, nasty, helpful female” genes in the population. Females that aid their mates therefore have an adaptive advantage.

Potential sources of error in the study include the difficulty of distinguishing males and females based on subtle morphological differences and the small size of the data set. These could be eliminated in future studies through sexing and marking of individuals and longer term observations. Yet the most important future research of *J. spinosa* would not deal with behavioral differences between males and females directly, but instead attempt to determine how and why polyandry developed in the species in the first place. Polyandry is, after all, an extremely rare mating system. In most species, including our own, males are the larger, more aggressive, and more promiscuous sex. In learning how and why *J. spinosa* females took on these traits, we may be able to understand how and why males of *Homo sapiens sapiens* and other species did as well. Such understanding

may be the key to explaining –and preventing– domestic violence and other such gender-based aggression in humans.

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Table 1. Frequency of aggression of *Jacana spinosa* females at the Palo Verde Marsh, Guanacaste Province, Costa Rica.

Female	Freq. of Aggression Against Females	Freq. of Aggression Against Males	Total
1	3	5	8
2	2	3	5
3	2	2	4
Total	2.3	3.3	5.7

Table 2. Frequency of aggression of *Jacana spinosa* males by female territory at the Palo Verde Marsh, Guanacaste Province, Costa Rica.

Female Territory	Males	Aggressive Acts	Frequency of Aggression per Male
1	5	13	2.6
2	3	8	2.7
3	3	4	1.3
Total	11	25	2.3

The effect of wetland management on bird species richness and diversity

Category: Independent Project
Participants: Nicole Donovan and Sarah Huber
Site: Palo Verde
Key words: Birds, species diversity, *Typhus domiguensis*, wetland management

Introduction

Palo Verde National Park, located in northeast Guanacaste, Costa Rica, remained undisturbed wetlands and tropical dry forest throughout the nineteenth century (Eugenio Gonzalez, pers. com.). Heavy grazing of the wetlands began in 1920, when a large portion of the area was converted into a cattle ranch (Gill 1988). OTS signed a lease on the property in 1970, but it was not until 1977 that government officials declared the surrounding area a wildlife refuge (Gill 1988). The primary goal of the refuge was to protect the large number of birds native to the region (E. Gonzalez, pers. com.). Though cattle were permitted to continue grazing throughout the refuge, hunting was banned (Gill 1988). In 1981, officials prohibited the grazing of cattle, and within two years cattails (*Typha domiguensis*) became the dominant species of the marsh (Gill 1988). Five years later, *T. domiguensis* had completely invaded the marsh. The establishment of a *T. domiguensis* monoculture led to a decrease in avian faunal diversity (Gill 1988). A program of controlled cattle grazing, implemented in 1987, aims to increase bird diversity by removing the cattail monoculture (Gill 1988). A dry season study has pointed to successful conservation efforts, with increased avian

diversity found in grazed areas (Manvill and Tossas 1997).

We have focused our investigation on the effectiveness of this wetland management in Palo Verde during the wet season, examining the difference in bird species richness and diversity between adjacent grazed and ungrazed areas of the marsh. Since wetland management (cattle grazing) has kept cattail populations to a minimum and increased dry season bird diversity during the past ten years, we hypothesized that a grazed section of the marsh would yield both a higher species richness and diversity of birds compared to an ungrazed section.

Methods

To investigate differences in bird species richness and diversity between managed and unmanaged wetland areas, observations were made from a tower located 50 m south of the air strip in Palo Verde National Park. Eugenio Gonzalez, director of the OTS biological field station at Palo Verde, aided in choosing the two areas of study (grazed and ungrazed), which comprised a total of approximately 30,000 m² of marsh, directly south of the tower. Both areas were estimated to be 100 m by 150 m, with the ungrazed area located 10 m east of the grazed area. This ungrazed section, adjacent to the grazed area, had its western border approximately

50 m east of the tower. The ungrazed section was comprised of 5% open marsh, 5% *Parkinsonia aculeata* (Palo Verde trees), and 90% *T. domiguensis* (cattails), while the grazed section contained 80% open marsh, 10% *P. aculeata*, and 10% *T. domiguensis*.

Observations were made from 5:40 to 6:40 am and 4:00 to 5:00 pm on November 5 and from 5:35 to 7:05 am and 4:00 to 5:00 pm on November 6. During each observational period, each of 2 observers used binoculars to conduct 5 minute scans, alternating areas every 5 minutes. Both observers would start by viewing the grazed area. In a 5 minute scan, each species observed was recorded. The number of sightings of each species in the 5 minute period was also recorded. At the end of 5 minutes, the two observers would follow the same procedure for the ungrazed area. Identification of birds followed Stiles and Skutch (1989).

A species-time curve was derived to ensure adequate observational time and Shannon-Wiener Index (H' : Meffe and Carroll 1997) was used to calculate species diversity of grazed and ungrazed areas. Species diversity (H') was also calculated without Northern Jacanas, as these birds represented 61% of grazed area sightings. T-tests (Magurran 1998) were calculated to test for significant differences between species diversity of grazed and ungrazed areas, both with and without Northern Jacanas. T-tests were also used to compare bird species diversity in grazed and ungrazed areas between our wet season data and dry season data of Manvill and Tossas (1997).

Results

Species-time curves containing total observation time (135 minutes) for grazed and ungrazed areas both plateau within approximately 100 minutes (Figure 1). The curve for the grazed area contains a higher plateau as a result of a greater species richness. The steeper slope of the grazed curve indicates that a shorter amount of time is needed to observe a greater amount of species in the grazed area compared to the ungrazed area (Figure 1).

Species-time curves reveal consistently higher species richness in the grazed area for each observed period. Data collected on November 6 from 5:35 to 7:05 am, however, yielded higher species richness in the ungrazed area (Figures 2 and 3).

There were 12 different species observed in the grazed area, with a total of 548 sightings. In the ungrazed area, 10 species were recorded, containing a total of 86 sightings (Table 1). Bird species diversity in the grazed area was significantly lower than bird species diversity in the ungrazed area ($H'_{\text{grazed}}=0.552$, $H'_{\text{ungrazed}}=0.804$, $t=6.48$, $df=119$, $P<0.001$). The Limpkin, Inca dove, and Spotted sandpiper were unique to the grazed area, while an unidentified species and Dove species were unique to the ungrazed area (Table 1). Northern Jacanas dominated sightings in the grazed area, representing 62% of total grazed sightings. Jacanas and Groove-billed Anis dominated the ungrazed section, with 23 and 22 respective sightings.

Bird species diversity excluding Jacanas resulted in no significant difference between grazed and ungrazed areas ($H'_{\text{grazed}}=0.686$, $H'_{\text{ungrazed}}=0.753$, $t=1.236$, $df=131$, $P>0.05$). In grazed areas, bird species diversity determined by Manvill and

Tossas(1997) for the dry season was found to be significantly higher than for wet season data presented in this study ($H'_{\text{dry/grazed}}=0.654$, $H'_{\text{wet/grazed}}=0.552$, $t=2.15$, $df=127$, $P<0.05$:). Conversely, in ungrazed areas, bird species diversity calculated by Manvill and Tossas (1997) was significantly lower for the dry season than for the wet season data presented in this study ($H'_{\text{dry/ungrazed}}=0.238$, $H'_{\text{wet/ungrazed}}=0.804$, $t=6.89$, $df=36$, $P<0.001$).

Discussion

Collected data refute our hypothesis that bird species diversity is greater in the grazed area than the ungrazed area. A significant difference was determined between bird species diversities of both areas, favoring a greater bird diversity in unmanaged wetlands. A possible explanation for decreased avian diversity in the grazed (managed) area may be a result of the large number of Jacana sightings, which represented over 60% of the observed sightings in the grazed area. When bird species diversity excluding Jacana sightings was calculated for both areas, no significant difference was found between bird species diversity for the grazed and ungrazed areas. By removing the large percentage of Jacanas, species evenness for the grazed area increases, therefore producing a greater species diversity.

Another possible explanation for a decreased species diversity of birds in the grazed area may be a result of seasonal effects on bird species diversity in the wetlands. Overall avian faunal diversity is known to decrease in the wet season due to insufficient resources (E. Gonzalez, pers. com.). Manvill and Tossas (1997)

examined bird species diversity in wetlands during the dry season. Dry season results (Manvill et al. 1997) yielded a significantly higher bird species diversity for the grazed area and a significantly lower bird species diversity for the ungrazed area, compared to our calculated bird species diversities for the wet season. Observations collected during the wet season may represent maximum bird species diversity for ungrazed areas and minimum bird species diversity for grazed areas, accounting for our higher overall bird species diversity of the unmanaged area. Greater resources available in the wetlands during the dry season attract migratory birds that increase bird species diversity in open, grazed areas (E. Gonzalez, pers. com.). The dry season may also cause an increased diversity in grazed areas, as bird species do not need to rely on tall vegetation, dominant in ungrazed areas, for refuge from harsh weather.

Though the ungrazed area contained a higher diversity of bird species, Purple Gallinules, Northern Jacanas, Groove-billed Anis, and Bare-throated Tiger-herons sighted in the ungrazed area inhabited open marsh areas representative of the grazed area. Thus, certain species contributing to an increase in ungrazed bird species diversity are potentially utilizing the small, rare, open marsh habitats similar to those composing a large percentage of managed areas. A decrease in visibility of the ungrazed area, resulting from the dense cattail cover, may have also skewed potential sightings. Large populations of certain species may have dominated small, open areas within the ungrazed area which were not visible from the tower. Thus, lower and more even species abundance yielding a higher species evenness in

ungrazed areas, may be a direct result of poor observation visibility of the ungrazed area.

Though the species-time curve shows adequate observational time was conducted in both areas, interesting trends are seen in the curves representing observational time during the rain. Species richness was found to increase in the ungrazed area during periods of bad weather, while grazed areas continually contained higher species richness values for periods without rain. Birds may use cattail cover as a refuge from harsh weather, escaping the open areas of grazed marsh during heavy wet season rains. Future studies should examine bird species diversity in managed and unmanaged areas in comparison to weather, as well as in comparison to wet and dry seasons.

Determining the effectiveness of management remains dependent on the definition of diversity. The Shannon-Wiener and Simpson indices represent fractional abundance of species, while other measures can be weighted by species importance, productivity, or size (Meffe and Carroll 1997). Though the managed area contained a greater species richness, the unmanaged area contained a higher species evenness. As cattle grazing has been found to increase the total number of species in the managed area, many conservationists may argue against management effectiveness, as evenness is not being maintained. As the measure of diversity

varies, conservation goals must become more precise. We must continue to examine wetland ecosystem requirements in order to determine opportune levels of species richness and diversity, as well as appropriate measures of this diversity, essential to the proper functioning and maintenance of the system.

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Table 1. Species richness and total number of sightings of each bird during 5 min scans of grazed and ungrazed areas of the wetlands in Palo Verde National Park, Costa Rica. A total of 135 min were spent scanning each area.

Species	Grazed	Ungrazed
Bare-throated Tiger-heron (<i>Tigrisoma mexicanum</i>)	11	6
Green-backed (Green) Heron (<i>Butorides striatus virescens</i>)	3	1
Black-bellied Whistling-duck (<i>Dendrocygna autumnalis</i>)	99	1
Limpkin (<i>Aramus guarauna</i>)	4	0
Purple Gallinule (<i>Porphyryla martinica</i>)	1	4
Northern Jacana (<i>Jacana spinosa</i>)	338	23
Spotted Sandpiper (<i>Actitis Macularia</i>)	4	0
Inca Dove (<i>Columbina Inca</i>)	1	0
Dove sp.	0	3
Groove-billed Ani (<i>Crotophaga sulcirostris</i>)	33	22
Hummingbird sp.	3	0
Tropical Kingbird (<i>Tyrannus melancholicus</i>)	42	16
Great Kiskadee (<i>Pitangus sulphuratus</i>)	9	9
Unidentified	0	1

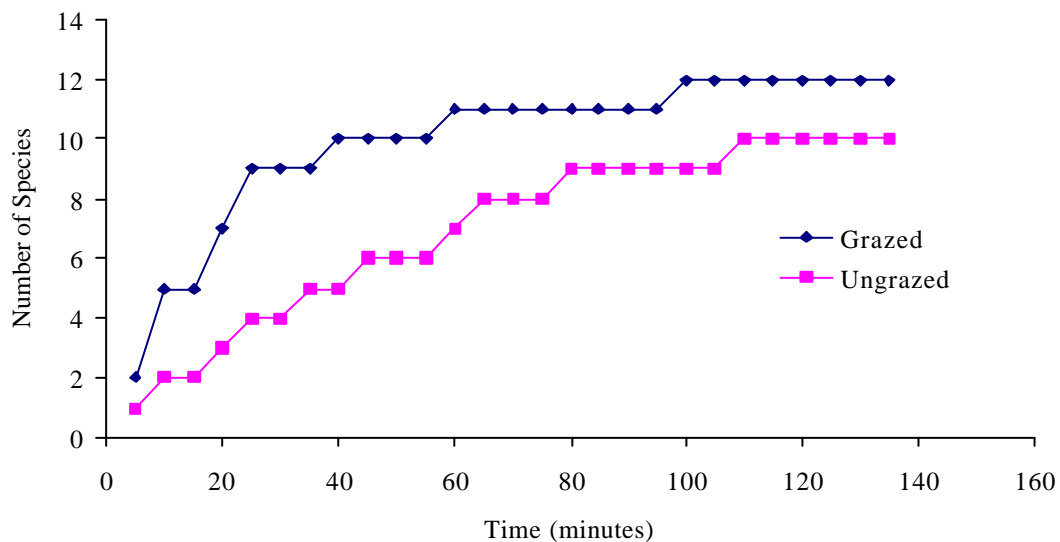


Figure 1. Species-time curve for observed birds in wetland areas in Palo Verde National Park, Costa Rica. The number of species was determined during five minute scans of grazed and ungrazed wetland areas. A total of 135 minutes were spent scanning each area.

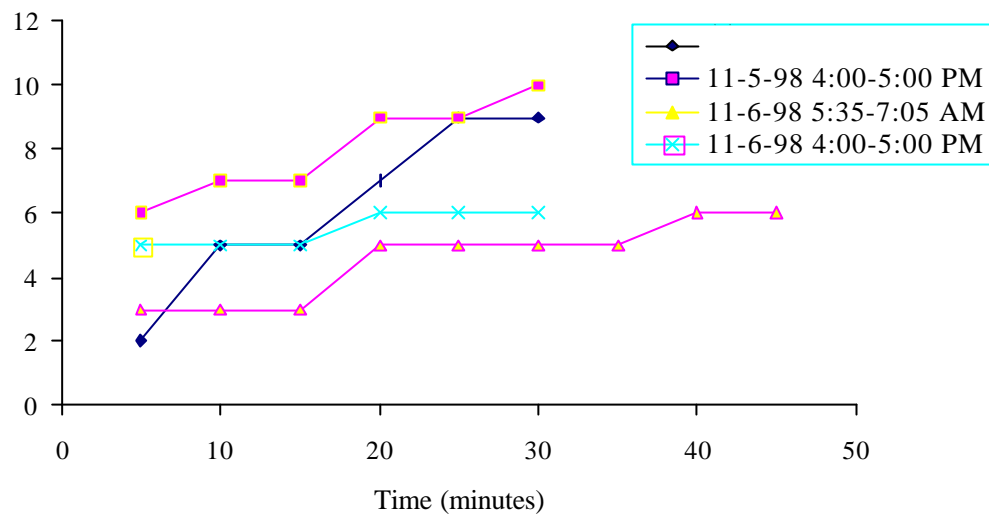


Figure 2. Species-time curve for observed birds in grazed wetland areas in Palo Verde National Park, Costa Rica. The number of species was determined during five minute scans of grazed wetland areas. A total of 30 minutes were spent scanning during three observations and 45 minutes were spent scanning during one observation.

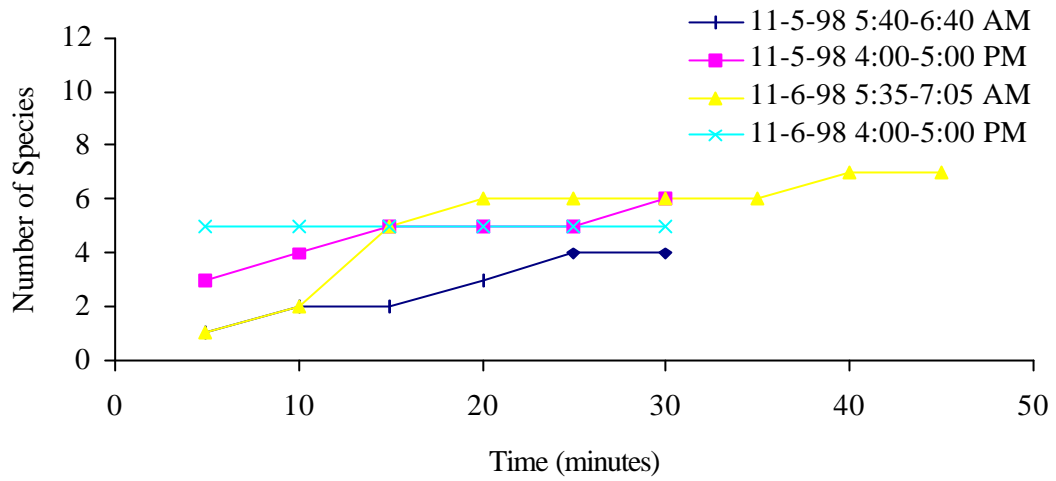


Figure 3. Species-time curve for observed birds in ungrazed wetland areas in Palo Verde National Park, Costa Rica. The number of species was determined during five minute scans of ungrazed wetland areas. A total of 30 minutes were spent scanning during three observations and 45 minutes were spent scanning during one observation.

LA SELVA

Foraging behavior and response to nectar availability of *Amazilia tzacatl*

Category: Independent Project
Participants: Serena Black and Jessica Lynch
Site: La Selva
Key Words: *Amazilia tzacatl*, behavior, forage, nectar

Introduction

Many plants produce sugary nectar to attract pollinators. Nectar constitutes an energy rich floral reward for the pollinator and benefits the plant through increased pollen exchange and reproduction (Kearns and Inouye 1993). Hummingbirds are important pollinators and major nectar consumers in the Neotropics. The high metabolism of hummingbirds, an adaptation for rapid flight, necessitates consumption of large nectar quantities. Meeting energy needs requires a balance between maximizing nectar intake and minimizing feeding time, energy spent foraging and defending territories (Temeles pers. comm.).

Because nectar is a valuable and often limited resource, many hummingbirds establish territories and defend flower patches against intruders. Territorial defense is most beneficial when resources are available at intermediate levels (Stiles and Wolf 1970). When nectar levels are low, the energy costs of defense outweigh the benefits of nectar intake. At high levels there is enough nectar for many visitors, and defense is not profitable from an energetic standpoint.

This project examined the effects of changing nectar availability on foraging and territorial behavior of the rufous tailed hummingbird, *Amazilia tzacatl*. We hypothesized that nectar volumes in flowers would decrease throughout the morning as

pollinators depleted floral rewards. We did not expect nectar concentration to change because amount of sugar in solution is independent of volume. Weather was humid and rainy at the time of our study and desiccation was not a factor influencing concentration. We predicted that foraging hummingbirds would visit more flowers per second through the morning as nectar levels declined. Hummingbirds were expected to maximize energy intake by exploiting higher quantities of flowers when nectar volumes are low. We also expected that territorial defense, measured by incidence of intruder chases, would be highest at mid-morning intermediate nectar levels.

Methods

Amazilia tzacatl is the most abundant Costa Rican hummingbird, from sea level to 1850 m, on both slopes (Stiles and Skutch 1989). *Amazilia tzacatl* prefers non-forest habitats and feeds on a wide variety of flowers (Stiles and Skutch 1989). *Amazilia tzacatl* is highly aggressive and often defends territories around rich clumps of flowers (Stiles and Skutch 1989). We chose *Amazilia tzacatl* as our study subject, because it is a common species and the hummingbirds were easy to find and observe over multiple days.

We chose *Stachytarpheta jaimecensis*, as our study plant because it produces abundant, densely clustered

flowers and provides rich resources for hummingbird foraging. *Stachytarpheta jaimecensis* is a member of the large Verbenaceae family. Cultivated for ornamental value, *Stachytarpheta jaimecensis* is an introduced flowering shrub in the Sarapiquí region, Costa Rica. Flowers are irregular and bisexual, with a tubular, five lobed corolla, and are arranged in racemose inflorescences (Heywood 1993).

Our project was conducted on December 1, 3-5 at La Selva Biological Station, Sarapiquí, Costa Rica. We observed hummingbird behavior between 6:00 and 10:00 am. Our study site included four *S. jaimecensis* bushes planted along the driveway near the comedor. Rain, broken by one clear morning on December 3, was the prevailing weather condition.

Nectar measurements

We used 2 μ L pipettes, inserted to the base of the corolla, to extract nectar from flowers. We calculated volume (μ L) by measuring length of nectar (cm) in the pipette, dividing by total pipette length, 3.2 cm, and multiplying by 2 μ L. To measure concentration of sugar we blew the nectar from the pipette onto the surface of a Sugar/Brix refractometer manufactured by Sper Scientific, Scottsdale, Arizona, USA. When the volume of nectar was too small for accurate measure, we diluted the nectar in the pipette with water and used the refractometer to analyze the dilute solution. Sugar content was determined by multiplying the concentration of dilute solution by the ratio between volume of dilute solution and nectar volume. Concentration was read in mg sugar/ml solution.

We measured changes in nectar volume and concentration throughout the

morning in unbagged flowers. At one-hour intervals between 6 and 9 am on December 3-5, we sampled nectar from five flowers each from a different location on the four bushes. We did not take nectar samples on the first day of observation, December 1, because we were perfecting our techniques and did not want to disturb the birds. On the following three days only five flowers were sampled per hour to minimize disturbance on hummingbird behavior. Flowers chosen were all dark purple, a sign of newly opened flowers, and had no small holes at base of corolla, a sign of nectar robbery by insects.

We also bagged three stems of flowers on the three separate days, marking the old or already opened flowers to avoid confusion, and left them overnight. We measured nectar in 3 undisturbed, newly opened flowers, one from each stem, the following morning. The values obtained provided estimates of nectar volume and concentration in new flowers.

Also, to test if flowers replenished nectar over time, we drained nectar from 8 flowers distributed on three stems, bagged them overnight, and used pipettes to check for nectar the following morning.

Hummingbird observations

To measure foraging behavior we timed feeding in seconds and counted number of flowers visited. We began timing as soon as we spotted a hummingbird feeding and continued until the hummingbird stopped feeding or left our view. We recorded frequency of territorial chases on all four days of observation to investigate whether there was a relationship between resource defense and nectar availability.

Only on one day were we able to collect accurate data on number of foraging bouts. On December 3, we recorded

number of feeding trips or bouts in ten-minute increments. December 3 was the one clear day without rain in our study period and the birds were more visible. On the other three days we were unable to collect complete observations of foraging bouts because the birds fed more frequently on the inside branches of the bushes.

Finally, to see if hummingbirds could recognize flowers that were new or previously unexposed to foraging later in the morning, we bagged a clump of twenty-one stems of flowers growing in close proximity on one bush, in the afternoon on December 4. At 8:30 am the following morning we removed the bags and exposed the new flowers to hummingbird foraging and observed until 9:30. We recorded number of previously bagged flowers visited per second during foraging bouts. For comparison, we also recorded number of flowers visited per second when the birds foraged in the previously unbagged area.

Analysis

We analyzed number of flowers visited per second, number of chases, number of foraging bouts, volume and concentration of nectar versus time of day by linear regression. We used 2-way Anova to compare number of flowers visited per second vs. time among the days and to compare nectar volumes vs. time among the days.

We performed a t-test comparing flowers versus visited per second in bagged and unbagged treatments to determine if foraging behavior differed between the two types.

Results

Nectar resources

We found no significant difference in nectar volume over time on December 3 ($R^2 = 0.07$, $F = 1.33$, $P = 0.50$), December 4 ($R^2 = 0.05$, $F = 1.02$, $P = 0.33$) or December 5 ($R^2 = 0.03$, $F = 0.47$, $P = 0.50$). A two-way Anova suggested that neither day nor time of day can explain the variance in nectar volume ($F = 0.64$, $P = 0.43$; $F = 0.01$, $P = 0.92$). We also found no significant difference in nectar concentration over time on December 3 ($R^2 = 0.02$, $F = 0.23$, $P = 0.64$), December 4 ($R^2 = 0.12$, $F = 2.03$, $P = 0.17$) or December 5 ($R^2 = 0.0008$, $F = 0.01$, $P = 0.93$). Nectar volume and concentration were unrelated on December 3 ($R^2 = 0.07$, $F = 1.31$, $P = 0.27$), Dec. 4 ($R^2 = 0.36$, $F = 10.29$, $P = 0.09$) and Dec. 5 ($R^2 = 0.002$, $F = 0.03$, $P = 0.86$).

Although small sample sizes precluded statistical analysis, we found a wide range in average nectar volumes and concentrations in bagged, newly opened flowers among the three days. Mean nectar volumes/flower declined three-fold from December 3 to December 5 (1.50 μL / 0.44 μL). Between December 4 and December 5, nectar concentration decreased by fifty percent (21.07 / 13.6 mg sugar / ml solution). We found no nectar in any of the flowers drained and bagged overnight to test if nectar was replenished.

*Foraging and territorial behavior of *Amazilia tzacatl**

Differences between number of flowers visited per second and time of day were significant for all four days of observation (Table 1, Figures 1-4). On each day average number flowers visited per second by the hummingbirds increased over time. For example, on December 3 average number of flowers visited per second increased from 0.81 from 6:00-6:30

to 1.16 between 8:30 and 9:00. Results of the two way Anova test suggest that time of day explained a significant amount of the variance in the number of flowers visited per second but day did not (Table 2).

The number of foraging bouts and time of day were unrelated on Dec. 3 ($R^2 = 0.015$, $F = 0.20$, $P = 0.66$). Chase frequency was negatively correlated with time of day on December 3 ($R^2 = 0.30$, $F = 5.53$, $P = 0.04$). Twenty-one chases were observed from 6:00 to 6:30, but only 8 were observed from 8:30 to 9:00. On December 4 and 5 there was no significant relationship between number of chases and time of day ($R^2 = 0.02$, $F = 0.40$, $P = 0.54$; $R^2 = 0.11$, $F = 2.46$, $P = 0.13$).

There was a significant difference in number of flowers visited per second between previously bagged and unbagged treatments ($df = 23$, $t = -10.35$, $P < 0.0001$). Hummingbirds visited less than half as many flowers per second in the area that had been bagged than in other areas of the bushes between 8:30 and 9:00. In the hour of observation hummingbirds visited an average of 0.59 previously bagged flowers per second ($n = 6$, $SD = 0.05$). In contrast, hummingbirds visited an average of 1.22 previously unbagged flowers per second within the same hour ($n = 19$, $SD = 0.03$).

Discussion

As hypothesized, on each of the four days of observation, we found that the number of flowers visited per second by hummingbirds increased over the course of the morning. Also consistent with our hypothesis, we found that hummingbirds visited fewer previously bagged flowers per second than unbagged flowers in foraging bouts within the same period of the

morning. Newly opened flowers, previously unexposed to foraging, appeared to be more valuable and worthy of energy expenditure than flowers already exposed over time. Results suggested that hummingbird foraging behavior was influenced by time and exposure of flowers to foraging. Observations from our study were supported by research conducted by Shelly et al. (pers. comm.) who demonstrated that bees spent more time at previously bagged flowers that had higher pollen supplies.

Contrary to our expectations, nectar volumes did not decrease over time on any of the three days that we sampled. However, measurements of nectar volume may have been misleading because we only sampled nectar in unrobbed flowers. Nectar in unrobbed flowers is depleted by hummingbirds alone. Nectar in robbed flowers is depleted by hummingbirds and insects. We frequently observed bees and wasps robbing nectar by cutting holes created at the bases of corollas. As the morning progressed it became increasingly difficult to find unrobbed flowers for nectar samples. Hummingbird foraging behavior may not be influenced by own foraging and nectar depletion but instead by nectar robbery. Territorial defense against the wasps and bees was not observed. It's possible that the robbers are too abundant and energy costs would be too high for the hummingbirds to chase them. Robbers crawl to the base of the corolla and are small so they may be hidden from the birds view and escape defense. Wasps and bees are slow and spend more time at each flower and may not be considered a significant threat because their foraging behavior is so different from the hummingbirds. Finally, the wasps and bees may sting the birds

detering defense. We did observe a territorial chase of a hawkmoth that was flying quickly and visiting many flowers in a foraging pattern very similar to the hummingbirds. The similarities in foraging might trigger defense or the hawkmoth may be more thorough and drain more nectar than the robbers. Competitive pressure from nectar robbery could be examined by random sampling of flowers in larger quantities to measure nectar availability and incidence of robbery over time.

Measurements of nectar volume on December 4 and 5 may have been affected by weather conditions. It was raining and water drops tended to gather around the narrow corolla. Inserting the pipette into the corolla without introducing some water into the nectar sample was very difficult. Water contamination would increase volume leading to inaccurate analysis of nectar changes over time. Long periods of rain and very little sun may also have affected the plants ability to photosynthesize, decreasing the energy available for nectar production over time. Average nectar volumes in previously bagged, newly opened flowers declined by 71% over the three days of sampling. Declines in nectar production could have affected analysis of changes in nectar over time among the days.

We hypothesized that hummingbirds would exhibit strongest territorial defense, in the form of chases, when nectar was available at intermediate levels. On December 3 chase frequency

decreased throughout the morning. The hummingbirds invested progressively less energy in territorial defense over time. Behavior was inconsistent with nectar availability which did not change over time. Its possible that the birds had not been able to feed as frequently in the rain on the following morning and initially defended nectar resources strongly but eventually tapered off to normal territorial defense levels as the morning progressed and they had had plenty of time to feed. Our observations were conducted on a limited time scale and its possible that behavioral extremes, both very early and later in the day, were not accounted for in our analysis.

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Table 1. Summary statistics for number of flowers visited per second by *Amazilia tzacatl* vs. time of day at La Selva Biological Station, Costa Rica

	R^2	F	P
December 1	0.39	36.49	<0.0001
December 3	0.32	32.24	<0.0001
December 4	0.23	26.04	<0.0001
December 5	0.14	11.21	<0.0013

Table 2. Summary statistics from two-way Anova for day and time of day vs. number of flowers visited per second by *Amazilia tzacatl* over a period of four mornings at La Selva Biological Station, Costa Rica

	F	P
Day	3.44	0.0650
Time of day	62.59	0.0001

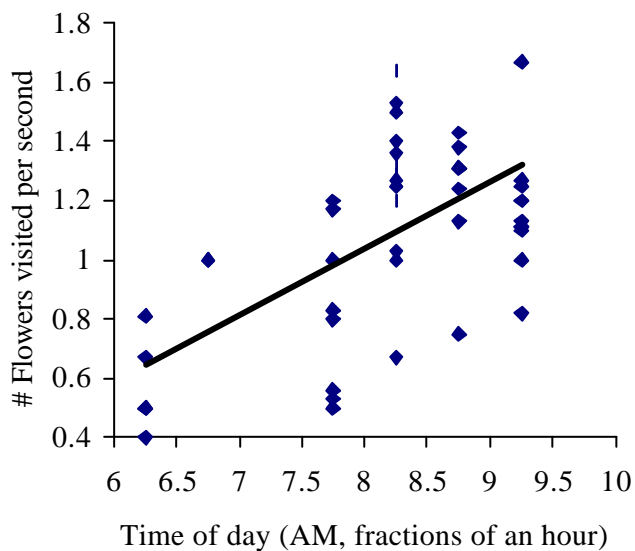


Figure 1. Number of flowers visited by *Amazilia tzacatl* per second vs. time of day on December 1, 1998, La Selva Biological Station, Sarapiquí, Costa Rica.

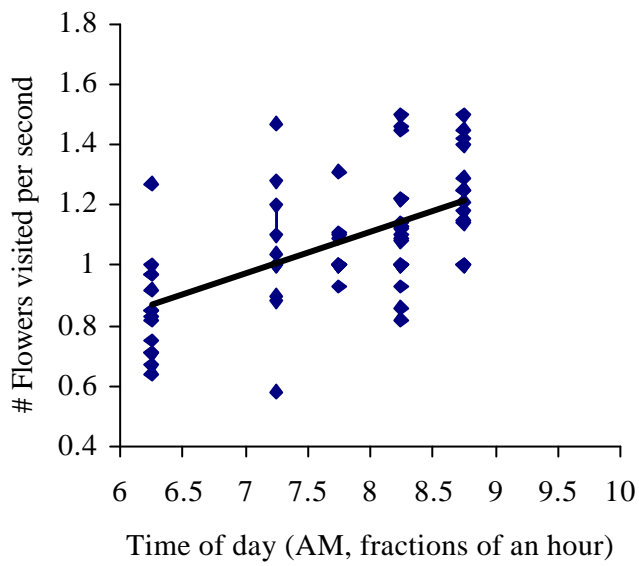


Figure 2. Number of flowers visited by *Amazilia tzacatl* per second vs. time of day on December 3, 1998, La Selva Biological Station, Sarapiquí, Costa Rica.

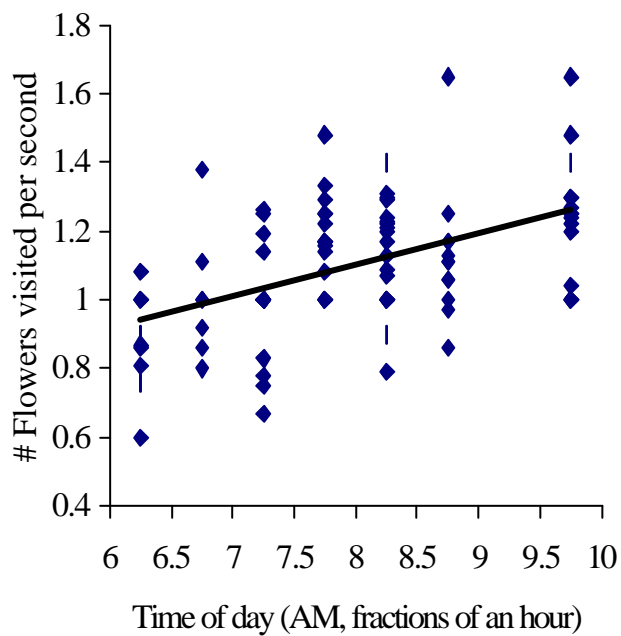


Figure 3. Number of flowers visited by *Amazilia tzacatl* per second vs. time of day on December 4, 1998, La Selva Biological Station, Sarapiquí, Costa Rica.

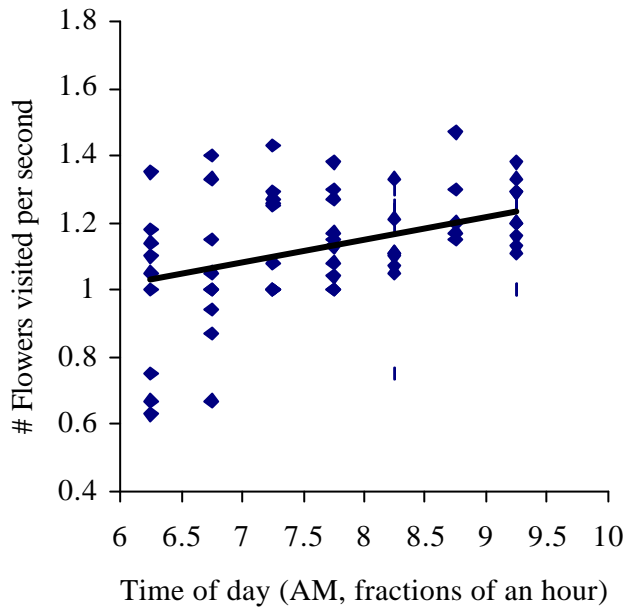


Figure 4. Number of flowers visited by *Amazilia tzacatl* per second vs. time of day on December 5, 1998, La Selva Biological Station, Sarapiquí, Costa Rica.

Spatial distribution and population sex ratio of a tropical tree *Neea psychotrioides*
(Nyctaginaceae)

Category: Independent Project
Participants: Brita Dempsey and Carl Salk
Site: La Selva
Key words: Dioecy, nearest-neighbor relationships, Monte Carlo simulation, *Neea psychotrioides*, sex ratios, spatial distribution

Introduction

Non-random spatial distributions of dioecious plants have been shown to increase pollen flow (Iglesias and Bell 1989) and to indicate specialized physiological or ecological requirements of the two sexes (Cox 1981). There are three possible spatial distribution patterns for the sexes of dioecious plants. Males and females can be positively associated (segregated or clumped by sex), negatively associated (nearest-neighbors are of unlike sex), or randomly distributed. Bierzychudek and Eckhart (1988) reviewed studies of 32 dioecious species and found that two thirds of them showed spatial segregation of the sexes (SSS). Although they admitted that this could be due to bias by biologists in their choice of organisms, they list a number of adaptive reasons for SSS including differential mortality, niche partitioning, and asexual reproduction (Bierzychudek and Eckhart 1988). Negative association is relatively rare and the mechanisms that produce it are still unknown but it is thought to increase pollination rates and fruit set (Iglesias and Bell 1989). Randomly distributed populations are neither positively nor negatively associated. They most likely exist where no selective pressures exist for the other, non-random, distributions.

Some of the mechanisms behind SSS can also be involved in causing population sex ratios to differ from unity. For example, differential mortality and niche partitioning could lead to higher survival rates or earlier maturation of one sex within a cohort (Opler and Bawa 1978). Different physiological requirements, especially in terms of reproductive investment, could result in one sex having more resources available for vegetative growth (Putwain and Harper 1972).

We investigated the spatial distribution and the population sex ratio of a small edge tree, *Neea psychotrioides* (Nyctaginaceae). To our knowledge the spatial distribution of this species has not been previously described.

Methods

This study was carried out at La Selva Biological Station in northeastern Costa Rica from 19 November to 2 December 1998. We censused *Neea psychotrioides* populations along Sendero Tres Rios (STR) and Sendero El Atajo (SAT). We attempted to locate trees by searching for foliage rather than fruits to avoid biasing our survey with the more conspicuous females (Wheelwright and Bruneau 1992). We marked every *N. psychotrioides* tree along the trails, determined its sex by the presence of fruit

or by flower dissection, and measured the distance between the base of its trunk and the base of the trunks of the nearest male (M) and female (F) trees. Three individuals without inflorescences were considered to be juvenile and not included in the census.

We calculated sex ratio and used a binomial test (Ott 1988) to test each population's deviation from a sex ratio of unity. We tested for non-random spatial distribution of the sexes in three ways. First, we compared the frequency of same-sex nearest-neighbors with the frequency of unlike-sex nearest-neighbors with what would be expected if the population were unsegregated, using a chi-square test (Pielou 1977, Magurran 1986). Second, we used the coefficient of segregation (Pielou 1977),

$$S = 1 - \frac{\text{Observed number of FM and MF pairs}}{\text{Expected number of FM and MF pairs}}$$

In a randomly distributed population, S would equal 0 and in a completely segregated population, S would equal 1. A negatively associated distribution would have S less than 0 (Wheelwright and Bruneau 1992). Lastly, to avoid the problems associated with non-independence of neighbors pairs, we used a Microsoft QBASIC (Version 1.1) program (Appendix) to perform a Monte Carlo simulation to test for negative association of the sexes. The simulation calculated the probability of observing more unlike sex pairs (MF and FM) if the population were completely randomly distributed. Each repetition of the simulation kept the population sex ratio and spatial distribution of the trees constant but randomly assigned the sex of each tree. The program was run 100,000 times and the number of times a

greater number of unlike-sex pairs than we observed was totaled and divided by 100,000 to calculate the probability of observing a more extreme negative spatial distribution by chance.

In addition to separate analyses by sub-population (STR, upper SAT, lower SAT, combined upper and lower SAT), we combined data from all populations to analyze overall trends.

Results

Of the 80 trees that we censused, 57 were female (71 %) and 23 were male (29 %). The male to female ratio was 1:2.54 and ranged from 1:1.75 to 1:12 in the sub-populations (Table 1). The binomial test showed that it is very improbable that the ratio for the total census would be observed by chance in a population whose sex ratio is 1:1 ($P < 0.0001$).

The sexes in all populations were randomly distributed (Table 1). Nearest-neighbors of like sex predominated overall as well as in each sub-population but not more often than expected by chance (Table 1). Coefficient of segregation values were closer to 0 than 1 which also supports the idea that the sexes are randomly distributed (Table 1). Finally, Monte Carlo simulations show that there is a very high likelihood of observing more unlike sex pairs in a randomly distributed population than this one (Table 1).

Discussion

The censused population of *N. psychotrioides* is strongly female-biased. We found a stronger female bias than Wolfe's (1997) study. She found the ratio to be 1 male : 1.54 females when she surveyed similar populations at La Selva in

1986-7. Opler and Bawa (1978) state that female-biased sex ratios could be caused by asexual reproduction, gametic selection, or differential mortality between the sexes. Asexual reproduction was not a likely cause for the observed skewed sex ratio as the spatial distributions of the sexes were not clumped. Gametic selection is thought to be dependent on pollination levels (Opler and Bawa 1978). To maximize fitness, a female receiving a high rate of pollination may adjust gamete ratios to produce an excess of female seeds. Unfortunately, we were unable to investigate pollination rates or seed production. Differential mortality is another possibility, but investigating this factor was beyond the scope of this study. Wolfe (1997) suggested that the higher rate of gall formation on male inflorescences could be so taxing that it puts the male trees at a distinct disadvantage and increases their mortality.

The sexes in the censused population were randomly distributed. It appears that *N. psychotrioides* does not show any signs of segregation, thus, it probably does not have niche differentiation either because of 1) similar physiological or ecological requirements for each sex or, more likely, 2) because the habitat that we surveyed was uniform. Both of these factors should be investigated before any definite determination of lack of selective pressures to cause non-random distribution is given.

Acknowledgements

O. Vargas helped us choose our study organism. Nicole, Greg, Carl, and Chris edited this paper. ☺ Thank you to Carl for reminding me time and again why we were doing something and for always sharing his granola. May he never need to

explain in Klingon that his hovercraft is full of eels.

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Table 1. Results from tests for spatial segregation of the sexes in populations of *Neea psychotrioides* at La Selva Biological Station, Costa Rica. See text for description of tests performed.

Population	X^2	P	S	Sex ratio Male: Female	Frequency of same sex pairs	Monte Carlo P
STR $n = 13$	0.09	0.9	0.08	1: 12.0	0.84	0.23
SAT $n = 67$	0.09	0.9	0.43	1: 2.04	0.58	0.55
Upper SAT $n = 44$	0.05	0.9	0.04	1: 1.75	0.52	0.33
Lower SAT $n = 23$	1.47	0.5	0.25	1: 2.83	0.69	0.76
Total $n = 80$	1.87	0.5	0.15	1: 2.54	0.62	0.71

Appendix

QBASIC program used to perform a Monte Carlo simulation to determine if the number of opposite sex nearest-neighbor pairs was greater than would be expected for a randomly distributed population. This example of the program was used for the STR subpopulation. Program code by C. Salk.

```

500 PRINT RND(1)
510 INPUT a
520 IF a = 3 THEN GOTO 970
530 GOTO 500

970 pval = 0
980 n = 0
990 prob = 0
1000 n = n + 1
1001 ssp = 0
1003 PRINT n; "th repetition"
1005 GOTO 1100
1010 NUA = RND(1)
1020 IF NUA < .923 THEN sex = 1 ELSE sex = 0
1035 REM .923 is probability of plant being female
1040 RETURN

1100 GOSUB 1010
1110 LET a = sex
1130 GOSUB 1010
1140 LET b = sex
1150 GOSUB 1010
1160 LET c = sex
1170 GOSUB 1010
1180 LET d = sex
1190 GOSUB 1010
1200 LET e = sex
1210 GOSUB 1010
1220 LET f = sex
1230 GOSUB 1010
1240 LET g = sex
1250 GOSUB 1010
1260 LET h = sex
1270 GOSUB 1010
1280 LET i = sex
1290 GOSUB 1010
1295 LET az = sex
1300 GOSUB 1010
1310 LET aa = sex
1350 GOSUB 1010
1360 LET ac = sex
1370 GOSUB 1010
1380 LET ad = sex

11300 LET females = a + b + c + d + e + f + g + h + i + az + aa + ac + ad
11310 IF females <> 12 THEN 1005

21000 ssp = 0
21005 REM ssp is the number of same sex pairs

```

```
21010 IF a = b THEN ssp = ssp + 1
21020 IF b = a THEN ssp = ssp + 1
21030 IF c = b THEN ssp = ssp + 1
21040 IF d = e THEN ssp = ssp + 1
21050 IF e = d THEN ssp = ssp + 1
21060 IF f = e THEN ssp = ssp + 1
21070 IF g = h THEN ssp = ssp + 1
21080 IF h = g THEN ssp = ssp + 1
21090 IF i = az THEN ssp = ssp + 1
21091 IF az = i THEN ssp = ssp + 1
21092 IF aa = az THEN ssp = ssp + 1
21094 IF ac = ad THEN ssp = ssp + 1
21095 IF ad = ac THEN ssp = ssp + 1

31499 PRINT ssp; "same sex pairs"
31500 IF ssp < 11 THEN prob = prob + 1
31505 PRINT prob; "instances of less than 11 same sex pairs"
31510 IF n > 99999 THEN GOTO 41600
31520 GOTO 1000

41600 pval = prob / 100000
41605 PRINT prob
41610 PRINT pval
41620 END
```

The role of drip tips in cleaning leaf surfaces

Category: Independent Project
Participant: Naamal De Silva
Site: La Selva
Keywords: colonization, drip tip, epiphyllae, leaf structure, leaf surface, rain forest, water runoff

Introduction

Drip tips, or the elongated apices of leaves, are virtually ubiquitous in wet lowland and lower montane tropical forests, but are far less common in drier or cooler ecosystems. Drip tips reach their peak abundance, diversity, and length in rainforest undergrowth. Because of this distribution, it has often been suggested that drip tips function in speeding and increasing water runoff from leaf surfaces. While there has been a great deal of speculation and controversy in popular and scientific literature on the role of drip tips, there have been few experimental studies on the function of drip tips (Dean and Smith 1978).

One of the most important functions of drip tips may be to channel rainwater from leaf surfaces, which can help remove debris, epiphyllae (epiphytes on living leaves), and fungi from leaf surfaces (Jungner 1891). In the humid environment of the rain forest, a wide variety of microepiphytes are abundant on living and dead plant material. Heavy rainfall and high biological productivity result in leaf litter falling onto leaf surfaces; soil can also splash up from the ground. These materials could reduce the photosynthetic capability of rain forest plants or facilitate colonization by parasitic epiphytes and fungi. As long as rainfall drains rapidly off leaf surfaces, it

could keep leaves relatively free of these substances.

In my study, I tested the hypothesis that drip tips can prevent or reduce the accumulation of epiphyllae, fungi, and debris on leaf surfaces. I also tested whether the type of cover varied due to the presence or absence of drip tips. Finally, I tested the assumption that the presence of a drip tip improves drainage of water from leaf surfaces, since this was the basis for my hypothesis on the function of drip tips in cleansing leaf surfaces.

Methods

I conducted my study between November 23 and December 4, 1998, in secondary growth rainforest at La Selva biological field station, in Sarapiquí, Costa Rica. La Selva lies in the northern Atlantic lowlands of Costa Rica, and receives approximately 4,000 mm of rain annually. I used 28 understory plants representing several species, all of which were woody shrubs or saplings. All study plants had simple leaves with entire margins and prominent drip tips (Figure 1). All leaves were similar in texture, shape, size, thickness, glossiness, and venation. The older leaves on the study plants all had some coverage by microepiphytes. To control for leaf age, and to use leaves free of epiphyllae, I used the youngest fully expanded leaves on each branch.

After locating and tagging the plants, I chose triplets of leaves, each of which received one of 3 treatments (Figure 1a): 1) removal of drip tips, leaving smooth, rounded leaf apices, 2) apices of leaves cut to resemble intact drip tips (to control for the effects of cutting), or 3) unmanipulated leaves. I cut the drip tips of the modified leaves so that they were approximately the same length as the drip tips on unmanipulated leaves. On each plant, I used either 3 or 6 leaves, depending on the availability of clean, fully expanded leaves. I used a total of 120 leaves, 40 of each treatment, on the 28 plants. For each leaf, I recorded measurements of leaf length, maximum leaf width, and drip tip length. I measured drip tip length as the distance from a leaf width of 1 cm to the apex of the leaf (Figure 1a).

On December 1 and 2, approximately 1 week after I set up the experiment, I recorded the amounts of accumulated substances on each of the leaves. I used a headlamp to examine the leaves, since the rainforest understory is always dimly lit. I classified the materials found on the leaf surfaces into the following categories: 1) epiphyllae (lichens, bryophytes, and algae), 2) fungi, and 3) debris (soil particles, leaf litter, and eggs). To estimate cover, I placed a transparent plastic sheet with a 5x5 mm grid of squares on each leaf, and counted the number of squares containing epiphyllae, fungi, or debris. I estimated total leaf area by counting the total number of squares that were at least half filled with leaf surface. I also noted the location and extent (high or low) of leaf damage due to herbivory, branch fall, and other factors.

Because of variation in leaf area, I used the measurements of the total number

of squares per leaf and of the number of squares of each type of cover to calculate % cover by type. I divided the number of squares containing epiphyllae, fungi, or debris by the total number of squares per leaf. To calculate total % cover, I added the values for the three types of cover and divided the resulting value by the total number of squares per leaf.

In addition, I recorded the angle of the drip tip, the angle of the leaf from base to apex, and the angle of the leaf from side to side. I used approximate categories of 30, 60, and 90 degrees from the plane of the ground.

On December 4, I tested the hypothesis that the presence of a drip tip increases water drainage from the leaf surface. For this test, I used 10 leaves of each treatment collected from 7 plants randomly selected from the original sample of 28. In the lab, I removed the leaves from their branches and stuck the petiole of each leaf into a base of molding clay, so that the blades were horizontal. After the leaf surfaces dried, I simulated light rain by dripping 5ml of water onto each leaf surface using a pipette, using 20-30 seconds to distribute the 5 ml over each leaf. I caught and measured the water that ran off the leaf with a 10 ml graduated cylinder.

I conducted all statistical analyses using JMP 3.22 (Sall and Lehman, 1996). I conducted a series of 2-way ANOVAs, and used pairwise contrast comparison tests to compare treatment means. To compare total % cover, I conducted a 2-way ANOVA including plant and treatment as effects. I also used 2-way ANOVAs each type of cover, with plant and treatment as effects. To test for variation due to leaf damage, I used a 2-way ANOVA comparing total % cover, with treatment

and % damage as effects. I did not statistically analyze variation due to leaf angle because of difficulties in collecting quantitative data (see Results). For the water drainage experiment, phenotypic variation among plants and differences in leaf size could affect the amount of water retained on leaves. Therefore, I used a 3-way ANOVAs comparing ml of water runoff, using treatment, plant, and leaf area as effects.

Results

There was abundant daily rainfall from November 23 through December 4. Fungi and epiphyllae rapidly colonized all leaves in the study. I observed visible amounts of colonization on November 30. However, even when I recorded measurements of % cover on December 1 and 2, all fungi and epiphyllae were small and did not cover much of the leaf area. Colonization by fungi was most rapid and widespread, both within and between leaves, with a mean (S.E.) of 15.8 (1.3) % cover over all three treatments. The prevalent type of fungi took the form of small black spots. These spots could be found anywhere on the leaf surface, but were usually concentrated along the mid-vein, at the base of the leaf (near the petiole), and along the drip tip. Epiphyllae were less common, with a mean (S.E.) of 6.3 (1.3) % cover over all three treatments. The most common epiphyllae were white, gray-white, or greenish white foliose lichens; bryophytes were also occasionally present. All epiphyllae were located to either side of the mid-vein, away from both the leaf base and the drip tip. Overall, there was not much debris on any of the leaves, with a mean (S.E.) of 2.6 (0.5) % cover

across treatments. Debris usually consisted of small dead leaflets and soil particles.

Leaves with cut off drip tips had a significantly higher mean total % cover than the other 2 treatments (Table 1, 2). Leaves with modified drip tips had a slightly lower mean % total cover than unmanipulated leaves, but this was not a significant difference (Table 1). Leaves with cut off drip tips also had a higher mean % cover of fungi ($F_{2, 90} = 7.9$, $P = 0.0007$, Table 1) than leaves of the other treatments, in spite of significant variation among plants ($F_{27, 90} = 3.7$, $P < 0.0001$). There was no difference in % cover by epiphyllae among treatments ($F_{2, 90} = 2.2$, $P = 0.11$, Table 1), but there was significant variation among plants ($F_{27, 90} = 1.9$, $P = 0.02$). Similarly, while there was no difference in % cover by debris among treatments ($F_{2, 90} = 1.3$, $P = 0.29$, Table 1), there was significant variation among plants ($F_{27, 90} = 4.1$, $P < 0.0001$).

There were very low levels of leaf damage during the week among leaves of all 3 treatments, with only 16 of the 120 leaves (13 %) experiencing low levels of damage, and 4 leaves (3%) experiencing high levels of damage. I found no significant variation in total % cover due to leaf damage ($F_{2, 115} = 0.3$, $P = 0.77$).

Leaf angle was somewhat constant among leaves of all treatments. Most plants hold their leaves horizontal in order to maximize light absorption. However, the angle of light hitting individual leaves can vary due to interference by other foliage, causing minor leaf angle differences among leaves. It was very difficult to take leaf angle measurements in the field to any useful degree of accuracy because the leaf surface tilted in many directions, and in 3 dimensions. Of the 120 leaves, 61 held

their leaves horizontal. However, only 27 of these leaves also held their drip tips at a 90° angle from the rest of the leaf, and did not tilt to the sides at all.

In the experiment comparing water drainage, leaves with drip tips cut off retained a significantly higher amount of water than the other 2 treatments (Table 1, 3). Modified drip tips retained slightly less water than unmanipulated drip tips, though this difference was not significant (Table 1). Modified and unmanipulated drip tips did not differ water in levels of runoff (Table 1).

Discussion

Overall, these findings supported my hypothesis that drip tips serve to reduce the accumulation of fungi, epiphyllae, and debris by increasing leaf drainage. This contradicts findings by Stahl (1893), Shreve (1914), and Seybold (1957), who argued that variation in accumulation of epiphyllae and debris was due entirely to microclimatic variation among plants. Even after controlling for variation among plants, I found that drip tips significantly reduce the chances of leaf surface colonization by fungi, and also improve water drainage.

Although % cover by fungi was significantly lower on leaves with drip tips, I found no differences in % coverage by epiphyllae. It is possible that epiphyllae were less restricted than fungi by the amount of water retained on the leaf surface. The water runoff experiment demonstrated that more water is retained on the surfaces of leaves without drip tips. Leaves without drip tips had higher rates of colonization by fungi. In addition, fungi were usually restricted to the mid-vein, leaf base, and drip tip. In both the field and the lab, I observed that these are the areas of the leaf surface that retain the most water

and take the longest to dry. Therefore, fungi, which seem very dependent on large, constant amounts of water, are more likely to colonize leaves without drip tips. Epiphyllae, which were almost always found on parts of the leaf surface that retain less water (i.e. the sides, away from main veins, the leaf base, and the apex), are probably independent of, or less dependent on retention of water on leaf surfaces. These epiphyllae grow in an extremely humid, rainy environment, and thus may not need require as much water as fungi. This would account for the studies of Stahl (1893), Shreve (1914), and Seybold (1957), who found that the presence or absence of drip tips on leaves had no effect on colonization by epiphyllae.

Also, the accumulation of debris did not vary by treatment. The added sheeting action of water due to the presence of a drip tip may not be enough to remove debris with large particle size, such as leaf litter.

I found leaf damage due to herbivory or other factors on few leaves. In the field, I observed that low levels of damage did not seem to have an effect on either colonization by epiphyllae and fungi, or on the accumulation of debris. By contrast, high amounts of damage appeared to greatly increase colonization by both fungi and epiphyllae. However, because only 3% of the leaves had high levels of leaf damage, it is difficult to generalize about possible effects leaf damage on colonization by fungi and epiphyllae without further research. Furthermore, it is likely that there would be different effects due to differences in the location and type of damage.

Leaf angle could cause large amounts of variation in % cover. A leaf held perpendicular to the ground would not

retain much water on its surface regardless of the presence of a drip tip. However, in this study, the seemingly low levels of variation in leaf angle were difficult to measure quantitatively.

Though the modified drip tip and the unmanipulated drip tip treatments were statistically not different, total % cover and % cover by type were lowest on the modified leaves. Similarly, the modified drip tip and unmanipulated drip tip treatments did not differ statistically in the water drainage experiment, but the modified drip tip leaves retained slightly less water on average than the leaves with unmanipulated drip tips. These trends support the idea that the modified drip tips were more effective in preventing or reducing colonization by fungi than are intact drip tips. In the field, and in the lab experiment, I observed that the modified drip tips were always straight, while the intact drip tips sometimes curved or twisted. It would be interesting to investigate the effects of drip tip twisting and curling further using both the % cover and the water drainage experiments, and comparing only modified drip tips and intact drip tips.

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Table 1. Means (S. E.) for total % cover, % cover by type, water drainage, and leaf damage for three treatments testing the effects of drip tips on the accumulation of fungi, epiphyllae, and debris on leaf surfaces. Means with the same letters are not significantly different at $\alpha = 0.05$ by a paired contrast comparison test. See methods of a description of the treatments.

	Unmanipulated	Modified drip tip	Cut off drip tip
% cover	0.22 (0.020) b	0.20 (0.020) b	0.32 (0.020) a
% fungus	0.14 (0.017) b	0.11 (0.017) b	0.20 (0.017) a
% epiphyllae	0.04 (0.014) a	0.07 (0.014) a	0.08 (0.014) a
% debris	0.03 (0.006) a	0.02 (0.006) a	0.04 (0.006) a
water runoff	4.78 (0.044) b	4.82 (0.044) b	4.60 (0.044) a
Leaf damage	0.23 (0.038) a	0.21 (0.038) a	0.33 (0.038) a

Table 2. Results of a 2-way ANOVA testing differences in total % cover by epiphyllae, fungi, and debris on the surfaces of leaves with cut off, modified, and unmodified drip tips; treatment and plant were included as effects.

Source	DF	SS	MS	<i>F</i>	<i>P</i>
Model	29	2.49	0.09	5.6	<0.0001
Treatment	2	0.36		11.6	<0.0001
Plant	27	2.14		5.1	<0.0001
Error	90	1.38	0.02		
Total	119	3.07			

Table 3. Results of a 3-way ANOVA comparing differences in water runoff captured (in ml) from the surfaces of leaves with cut off, modified, and unmodified drip tips; treatment and plant were included as effects.

Source	DF	SS	MS	<i>F</i>	<i>P</i>
Model	9	0.4	0.0	2.6	0.03
Treatment	2	0.3		6.9	0.01
Plant	6	0.2		1.5	0.23
Leaf Area	1	0.0		0.1	0.76
Error	20	0.4	0.0		
Total	29	0.8			

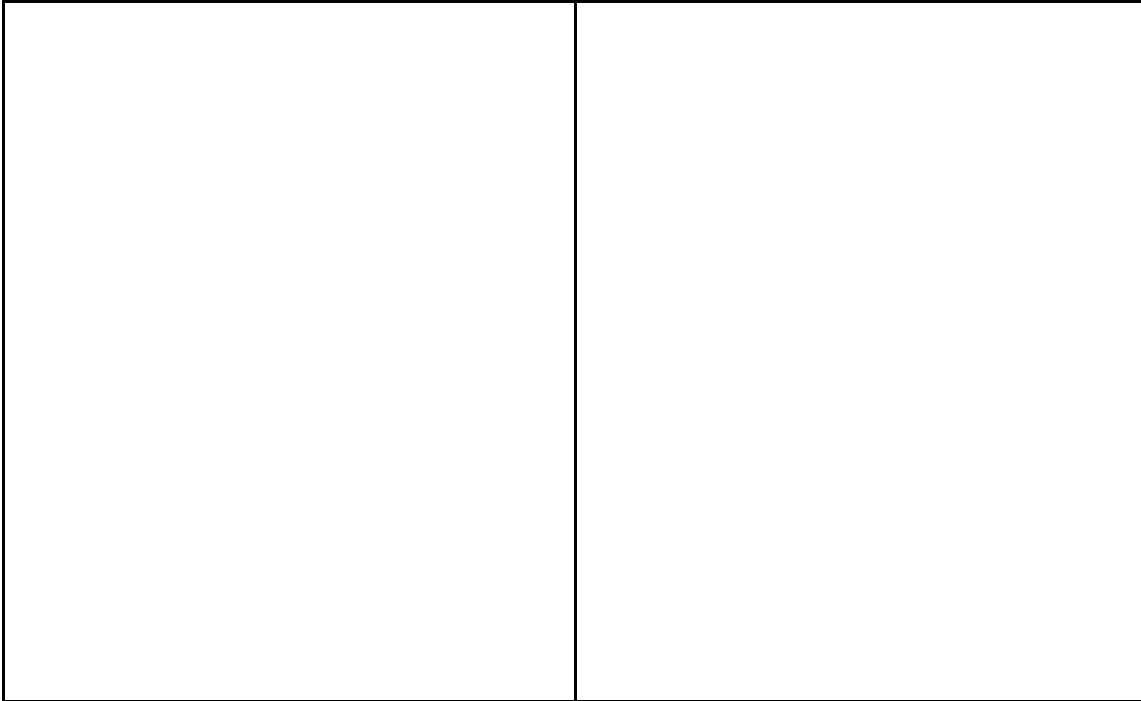


Figure 1. A) General leaf shape used, illustrating the three different treatments, where a = uncut drip tip, b = experimentally modified drip tip, and c = drip tip experimentally removed. B) General leaf shape used, illustrating usual locations of colonization by epiphyllae and fungi.

Effects of defecation strategy upon soil composition and dung visitation

Category: Independent Research

Participants: Brad Feldman

Site: La Selva

Key Words: *Bradypus variegatus*, defecation strategy, dung visitation, soil composition

Introduction

In tropical lowland forests mammalian dung is a nutrient-rich, limiting resource subject to fierce competition by dung beetles and flies (Sherman 1993). Although Janzen (1983) has stressed the value of studying patterns of dung input and uptake, these essential interactions have received minimal attention in the tropics. Input of mammalian dung into ecosystems occurs via a variety of defecation strategies. For example, a common primate defecation strategy (PDS) is to defecate from tree branches. This often creates the piles of dung on the forest floor as seen in areas of high primate density (Janzen 1983). In contrast, a feline defecation strategy (FDS) typically involves the burying of feces beneath dirt kicked up by the feline's hind legs (Deinert pers. comm.).

Perhaps most intriguing is the defecation strategy of *Bradypus variegatus* (SDS), the three-toed sloth. Exclusively an arboreal species, three-toed sloths descend to the forest floor about once a week to defecate. Prior to defecation, sloths dig a small hole in the ground with their tail. The feces are then deposited in this depression, urinated upon, and covered with leaves. This process takes nearly 30 minutes but represents a substantial risk to sloths. Sloths are well camouflaged in the trees where they are normally found but are exposed to a variety of predators on the ground. The foremost

explanation for ground defecation is that it evolved to increase the nutrient supply to trees on which sloths feed (Montgomery 1983). Thus, through its defecation strategy sloths increase the productivity of the trees from which it prefers to feed. This hypothesis assumes that buried sloth feces will undergo what Montgomery and Sunquist (1975) described as slow decomposition, wherein soil nutrient absorption is abnormally high.

This study examined the effects of the PDS, FDS, and SDS upon soil composition and insect visitation to dung. I hypothesized that feces deposited using a SDS would increase the nutrient content of the soil more than feces deposited using either the FDS or PDS. I further predicted that dung visitation would be greatest for feces deposited via a PDS and least for feces deposited via a SDS. This prediction was based on the assumption that it is difficult to locate and consume feces that are hidden beneath leaves or dirt. Urine may act to further decrease insect visitation by creating an environment around the feces which is either toxic or impenetrable to insects. Although it is possible that microbial consumption of feces may increase below ground, I assume that this consumption is negligible in comparison to the consumption by insects. Feces above ground should be easiest to find and consume. In developing a second prediction I made the assumption that dung visitation mirrored dung

consumption. Therefore, due to a low level of consumption, the soil beneath feces of a SDS would absorb a greater amount of fecal nutrients than the soil below feces of either a FDS or PDS.

Methods

I conducted this study from November 18 to December 2 1998 at La Selva Biological Station, Costa Rica. Six sites in the secondary forest were selected for ease of observation. At each site three points on the forest floor separated by approximately 1m were selected for fecal decomposition. Immediately adjacent to all selected fecal deposition points (DPs) I took soil samples from a depth of 5cm to 10cm below the ground.

I used exclusively human dung collected within 30 minutes prior to dung placement. Each fecal sample was partitioned into three pieces of 15cm³, an arbitrary volume resulting from limited dung availability. The remaining dung was discarded. I imitated a PDS by simply placing a piece of dung above the ground. In order to mimic a FDS I placed a piece of dung above the ground but then covered it with a 20cm² piece of mesh. This mesh was then covered with a thin layer of soil. Mesh was used as a device to facilitate spot check observations of the buried dung following dung placement. For the SDS imitation a rectangular hole with a 56cm² area was dug 10cm deep into the ground. Next I placed the dung in the hole, urinated upon it at full stream for 10 s, and covered it with 5-6 leaves. The actual sloth defecation strategy has not been described in detail, therefore hole size, urine volume, dung volume, and leaf number, were all best approximations from the literature previously cited.

Over the course of six different days I recorded insect visitation at six different sites. On a single day all observations were made at a single site. At each site I observed insect visitation to dung of each of the defecation strategies, PDS, FDS, and SDS. Dung of an individual defecation strategy was observed for 63.5 minutes. The sequence in which I deposited and observed dung of different defecation strategies was varied over the six days. These sequential observation periods were divided into 7 consecutive time intervals of the following duration (in seconds): 30, 60, 120, 240, 480, 960, 1920. At the termination of each interval I counted the number of dung visitors and then noted the species of these visitors.

I created the following five categories to facilitate insect visitation comparisons:

Recruitment Time (RT)- time elapsed before the first insect was seen on the dung during a spot check

Foraging Effort (FE)- sum total of visitors tallied during spot checks

Species Richness (SR)- total number of different morphospecies of visitors over the 7 spot checks

Average Diversity (AD)- mean number of morphospecies found at individual spot checks

Consumption Capacity (CC)- highest number of visitors found on dung over the 7 spot checks.

Results from these categories provide a general picture of the actual number,

potential number, and diversity of insect visitor to feces of the three defecation strategies.

Fifteen soil samples taken from a depth of 5cm to 10cm below each DP were collected 3-7 days after the first fifteen feces were deposited. The variation in collection time was purposeful because I did not know what amount of time would be most indicative of fecal nutrient effects upon the soil. Soil from below the DPs of an individual site was always collected on the same day. Time constraints did not allow me to collect soil samples from the sixth trial. I analyzed the ammonium and phosphate content of all soil samples using protocols from *Tropical Soil Biology and Fertility: A Handbook of Methods* (Anderson 1989). Ammonium and phosphate were chosen because they are essential nutrients for plant growth. Time constraints did not allow me to analyze all other essential nutrients in the soil. I calculated the percent change in soil composition for ammonium and nitrogen. This was done by subtracting the initial soil concentration from the final concentration, dividing this difference by the absolute value of the initial concentration, and then multiplying this quotient by 100.

The JMP program was used to analyze all data (Sall 1996). A Chi-Squared approximation of a Kruskal-Wallis was used to establish differences amongst the three defecation strategies in both the aforementioned visitation categories and the percent soil composition changes. A Turkey-Kramer test of multiple comparisons was used in order to establish differences between individual defecation strategies.

Results

I found significant differences in Foraging Effort, Species Richness, Average Diversity and Consumption Capacity between the three strategies. No difference was found in Recruitment Time between strategies (Table 1). The Species Richness of SDS was less than that of both FDS and PDS. However, there were no differences between SDS and FDS in FE, AD, or CC. Both SDS and FDS were different from PDS in FE, AD, and CC (Table 2).

No significant differences were found between the strategies in percent soil composition changes of either ammonium or phosphate content (Table 3). The means and standard deviations for percent soil composition change are given in Table 4. While no statistical differences were found between SDS and PDS in percent composition change, the percent phosphate change of SDS was on average nearly ten times the percent phosphate change of PDS. Further, the mean percent ammonium change of SDS was roughly three times the mean percent ammonium change of PDS.

Several morphospecies, including all observed beetles and ants, were never seen visiting the dung of the SDS. At the six sites the total number of different morphospecies seen on the feces of the PDS, FDS, and SDS varied. This variation is illustrated in Table 5.

Discussion

The hypothesis that a sloth defecation strategy would increase soil nutrient absorption more than other defecation strategies was not supported by the collected data. Therefore, this study does not support Montgomery's slow decomposition hypothesis. An alternate hypothesis may be that the sloth performs this intricate defecation strategy in order to

decrease predation by diminishing its fecal scent. Felines, animals with a well-developed sense of smell, are capable of climbing trees and can occasionally predate sloths (Deinert pers. comm.). This alternative hypothesis should be examined further.

Clearly both the feline and sloth defecation strategies decrease the diversity and number of insect visitors relative to the primate defecation strategy. There was also a decrease in total number of morphospecies seen on the dung of the SDS as compared to the dung of the FDS and the PDS. The effects of these decreases in visitation on insect reproductive success and insect resource competition have not been examined. Further, the differences found for dung insect visitation between FDS and SDS are not adequate to support the hypothesis that a SDS substantially decreases insect visitation.

Future studies should consider examining a larger sample size for more thorough comparison. The greatest increases in percent soil composition change were found in soil samples collected five days after fecal deposition. This indicates that when collections must be made with a week of fecal deposition, a five day period is optimal for fecal nutrient leakage into the soil. To most accurately characterize nutrient flow from dung, future studies should consider collecting all samples five days after deposition.

It remains certain that three-toed sloths undergo a considerable risk in implementing their defecation strategy. The hypothesis that this strategy is enacted in order to differentially support the growth of trees from which the sloth feeds is not supported by this paper. The other current

hypothesis, which suggests that this strategy is used to decrease predation on the sloth, is somewhat paradoxical in that the sloth must descend to the ground in order to avoid what are primarily terrestrial predators. Noting that the sloth's dung is home to many specialized insect larvae (Montgomery 1983), I propose a new hypothesis. Perhaps the sloth has evolved its defecation strategy as a way to diminish the number of insect larvae that can successfully parasitize the sloth. The first assumption of this hypothesis is that the insects living on sloths parasitize them. An individual sloth can be found with as many as 900 beetles on its coat, as well as mites and the Pyralid moth, or sloth moth (Montgomery 1983). Further studies are needed to examine the parasitic costs of these numerous insects on the sloth. The life cycle of these potentially parasitic insects involves first ovipositing in the sloth's dung, and then living on the sloth as an adult (Montgomery 1983). By urinating on its feces it is possible that the sloth creates an environment in which insect larvae are incapable of growing. In this study beetles were never seen on the dung of the SDS. This supports the idea that urine may create a toxic environment for some species. Further, by covering its buried feces with leaves a sloth may decrease the likelihood that rain will dilute the sloth's urine. This hypothesis would be difficult to prove, but initial examination would involve testing the ability of larvae to survive the harsh environment created by the sloth's urine.

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Table 1: Results from a χ^2 approximation of a Kruskal-Wallis test comparing Recruitment Time (RT), Foraging Effort (FE), Species Richness (SR), Average Diversity (AD), and Consumption Capacity (CC) among the three defecation strategies (Primate, Feline, Sloth)

Category	χ^2	d.f.	P
RT	10.819	2	0.0045
FE	13.203	2	0.0014
SR	7.799	2	0.0202
AD	12.682	2	0.0018
CC	10.529	2	0.0052

Table 2. Means (S.E.) of foraging effort (see text), insect species richness, average insect species diversity, and consumption capacity (see text) at three experimental defecation strategies. PDS = Primate Defecation Strategy; FDS = Feline Defecation Strategy, SDS = Sloth Defecation Strategy. Means with the same letter are not significantly different at $\alpha = 0.05$ by a Tukey-Kramer HSD test comparing defecation strategies.

	PDS	FDS	SDS
Foraging Effort (n=6)	45.333 (5.226)a	11.667 (3.827)b	2.667 (1.229)b
Species Richness (n=6)	4.333 (0.494)a	3.167 (0.703)a	1.500 (0.500)b
Average Diversity (n=6)	2.286 (0.188)a	0.810 (0.201)b	0.357 (0.164)b
Consumption Capacity (n=6)	13.667 (1.820)a	4.833 (1.195)b	2.500 (1.522)b

Table 3: Results from a χ^2 approximation of a Kruskal-Wallis test comparing percent soil ammonium and phosphate change among the Primate Defecation Strategy (PDS), Feline Defecation Strategy (FDS), and Sloth Defecation Strategy (SDS).

Category	χ^2	d.f.	<i>P</i>
Ammonium	1.3400	2	0.5117
Phosphate	3.500	2	0.1738

Table 4: Means and standard deviations of % ammonium and phosphate change for the primate defecation strategy (PDS), feline defecation strategy (FDS), and sloth defecation strategy (SDS). (n=5)

Defecation Strategy	% Phosphate Change	% Ammonium Change
PDS	538.45 (870.10)	339.61 (364.50)
FDS	-231.74 (529.04)	381.80 (621.95)
SDS	4922.40 (8715.66)	1019.36 (1856.31)

Table 5: Insect visitation to the Primate Defecation Strategy (PDS), Feline Defecation Strategy (FDS), and Sloth Defecation Strategy (SDS).. An X signifies that the insect was observed on the feces of the defecation strategy.

Identification	PDS	FDS	SDS
Sarcophagidae	X	X	X
Calliphoridae/metallic			X
Calliphoridae/black	X	X	X
Muscidae	X	X	
Sepsidae	X	X	X
Small Black Beetle	X	X	
Large Black Beetle	X		
Black Ant	X	X	

Wimps or weight-lifters: an examination of foraging efficiency in *Atta cephalotes*

Category: Independent Project
Participants: Kristen Ford and Andrew Knoll
Site: La Selva
Key words: Ant morphology, *Atta*, foraging behavior, leaf-cutter ants

Introduction

A walk through the rainforest always reveals a trail of countless numbers of leaf-cutter ants darting across the path. It is difficult to pass them without becoming increasingly curious about their foraging activities. Leaf-cutter ants of the genus *Atta* cut 12-17% of total leaf production from tropical forests (Cherrett 1986). They bring this vegetation back to their nests to culture fungus to use as food for their larvae (Holldobler 1990). Upon closer inspection of these ants, one may notice a wide range in worker size, leaf fragment size and speed of the workers. Larger ants are associated with quicker foraging (Cherrett 1986). Larger ants are also positively correlated with carrying larger leaf loads and are correlated with a higher maintenance cost (Futuyma et al. 1990). These observations led us to question the foraging strategy of *Atta* workers. Are larger ants the most efficient foragers? How much does the cost of nutrition change this efficiency? One would predict that evolutionary forces have selected for the individual ant morphology and behavior that would allow the greatest amount of biomass to be brought into the colony in the smallest amount of time. Individual ants must carry leaf fragments of a size that will optimize their foraging efficiency in light of the ant's own nutritional cost. In this study, we will examine this issue of foraging efficiency.

We determined ant sizes, leaf sizes and the speed at which ants were returning to the colony with their fragments. With this data, we examined the relationship between ant size class and foraging efficiency, which we defined as the amount of biomass carried into the colony over a given distance per unit time. To take this measure of efficiency a step further, we calculated the net energetic yield of each ant, which is the efficiency of the individual per nutritional cost to see if this measure differed among different ant size classes. We hypothesized that larger ants would demonstrate higher foraging efficiency because they should be able to carry heavier loads at a faster speed. We also predicted that the net energetic yield would be similar among the different size classes because this measure would take into account the higher nutritional costs of the larger workers.

Methods

We conducted our experiment on December 2 and 3, 1998 at La Selva Biological Station in Costa Rica. We examined a colony of *Atta cephalotes* to the right of the bike path near the visitor's center. We chose this colony because the ants' paths to the colony were well defined and we could easily determine a 1 meter section over which to track the ants. We first measured the ants' travel time over this one-meter path to the nearest one hundredth of a second. The 100 study ants

were chosen at random. After determining this velocity, we placed the ants and their leaf fragment in a jar and froze them so that we could measure leaf mass, ant mass and head width for each ant. We measured masses to the nearest 0.0001 g. We measured head width to the nearest 0.01 mm with an ocular micrometer. We used leaf mass, and ant velocity to obtain an efficiency value for each ant using the equation:

$$\text{Efficiency} = \text{leaf mass} \times \text{velocity}.$$

We used head width to determine the nutritional cost to the colony of each ant using the following modified version of the equation presented in Futuyma et al. (1990).

$$\text{Nutritional cost} = (\text{head width})^2.$$

We modified the original by substituting head width for body length. These measures are strongly positively correlated (Deinert pers.comm.). Net energetic yield was calculated as

$$E_{\text{net}} = \text{efficiency}/\text{nutritional cost}$$

To compare the foraging efficiency of different ant sizes we divided the ants into three size classes by their masses- < 0.004 g, 0.0041 g-0.0057 g, >0.0057 g. We also divided the leaf masses into two size classes- < 0.0107 g and > 0.0107 g. These categories were determined arbitrarily based on our normally distributed data. For the ant mass divisions, we divided the number of individuals by three and used the mass of the last individual in each group. For leaf mass, we divided the classes at the median mass. These divisions

allowed us to form six ant classifications: small ants carrying large and small leaves, medium ants carrying large and small leaves and large ants carrying large and small leaves.

We performed a chi square analysis to determine which sized ants carried different sized leaves. We used a one-way ANOVA to determine if a significant difference in efficiency existed between the different ant categories. We used the Tukey-Kramer test of multiple comparisons to compare the mean efficiency of each category with each of the others. We used a one-way ANOVA to determine if a significant difference in net energetic yield existed between the different ant categories. We used the Tukey-Kramer test of multiple comparisons to compare the mean net energetic yield of each category with each of the others (SAS Institute 1996).

Results

Our data revealed that in general, ants tended to carry leaf fragments proportional to their size ($\chi^2 = 6.71$, $df = 2$, $P < 0.05$). Our data revealed a significant difference in ant velocities among the different ant size categories (Table 1). Mean velocities were higher for ants carrying small leaves than for ants carrying large leaves and were higher for large ants than for small ants (Table 2). We found a significant difference among ant types with respect to efficiency (Table 3). Mean efficiencies were higher for ants carrying large leaves than for ants carrying small leaves and were higher for large ants than for small ants (Table 4). We found a significant difference among ant types with respect to net energetic yield (Table 5). A significant difference in mean net energetic yield was found between small ants carrying

small leaves and small, medium and large ants carrying large leaves. Significant differences in mean net energetic yield were not found between the other ant categories (Table 6).

Discussion

We predicted that individual ants would tend to carry leaf fragments of a size that will optimize their foraging efficiency in light of the ant's own nutritional cost. Many studies have found a strong positive correlation between the size of the ant and the size of their leaf load (Futuyma 1990). Large ants must carry loads that are large enough so that their efficiency is not hindered by their higher nutritional costs. In addition, before looking at efficiency, we wanted to see which ants foraged at a quicker pace. Our results showed that larger ants moved faster than smaller ants. Perhaps this energy expenditure will lead to a higher nutritional cost for larger ants than for smaller ants since workers expend up to seven times more energy while running than while resting (Nielsen et al. 1982).

Since larger ants in general carried larger leaves than smaller ants and since larger ants forage at a higher velocity than smaller ants we would expect larger ants to demonstrate higher efficiency than smaller ones. The one-way ANOVA confirmed this prediction. This finding leads us to ask an important question. If larger workers are more efficient foragers, then why does such a range of size classes exist? It would seem that the colony would have evolved to only produce larger ants.

We explored two possibilities to answer this question. One idea is that among one ant size class, velocity may decrease as mass of leaf load increases. The larger leaf loads carried by larger ants

may contribute to decreasing their efficiency. Our data reveal though that this is not the case. No significant difference exists between the velocity of ants of one size class carrying large and small leaf loads.

The other possibility is to add in the cost of nutrition as a factor to the efficiency measure because we believed that the larger ants may bring more biomass into the colony, but would cost more for the colony to maintain. We hypothesized that the significant differences that existed between ant types with respect to efficiency would not exist with respect to net energetic yield. For the most part our data revealed this to be true. The only ant class that demonstrated a significantly lower net energetic yield than the other classes was small ants carrying small leaves. Even though they have a low nutritional cost to the colony, they are not bringing in enough biomass in a quick enough amount of time to match the net energetic yield of the other workers. Perhaps these ants would be better suited to work in another aspect of colony life. However, significant differences did not exist in net energetic yield among any of the other size classes. The small ants carrying large loads and medium ants carrying small and large loads are making an equal contribution to the colony. The smaller ants' contribution is approximate to the large ants since larger ants have a higher nutritional cost than they do. This is an important finding because it helps to explain why the colony produces a wide size range of ant sizes.

There are further aspects of foraging behavior that would be interesting to explore. We did not measure cutting time or observe cutting behavior, which could have affected our observed measures

of efficiency. We also did not account for the quality of the leaf fragments. Our assumption was that more biomass was more beneficial to the colony without examining the quality of the different sized leaves.

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Table 1. Summary of ANOVA statistics for ant velocity among various ant-types

Source	DF	Sum of Squares	Mean Square	F
Model	5	0.00124	0.000248	8.2708
Error	94	0.00282	0.000030	$P < .0001$
C Total	99	0.00405	0.000041	

Table 2. Mean velocity (meters/second) of various ant-types.

Ant-Type (<i>n</i>)	Mean*	Std Error
Large ant, large leaf (23)	0.0285 ab	0.01681
Large ant, small leaf (11)	0.0336 a	0.02431
Medium ant, large leaf (15)	0.0250 bc	0.02082
Medium ant, small leaf (19)	0.0310 a	0.01850
Small ant, large leaf (12)	0.0225 c	0.02328
Small ant, small leaf (20)	0.0245 bc	0.01803

*Means followed by the same letter are not significantly different at $\alpha = .05$ based upon the Tukey-Kramer HSD Test of Multiple Comparisons.

Table 3. Summary of ANOVA statistics for efficiency among various ant-types.

Source	<i>DF</i>	Sum of Squares	Mean Square	<i>F</i>
Model	5	1.410	0.282	29.3571
Error	94	0.903	0.00961	$P < .0001$
C Total	99	2.313	0.0234	

Table 4. Mean efficiency (gram*meters/second) of the various ant-types.

Ant-Type (<i>n</i>)	Mean*	Std Error
Large ant, large leaf (23)	0.4832 a	0.02044
Large ant, small leaf (11)	0.2768 bc	0.02955
Medium ant, large leaf (15)	0.3485 b	0.02531
Medium ant, small leaf (19)	0.2389 c	0.02249
Small ant, large leaf (12)	0.3226 bc	0.02830
Small ant, small leaf (20)	0.1383 d	0.02192

*Means followed by the same letter are not significantly different at $\alpha = .05$ based upon the Tukey-Kramer HSD Test of Multiple Comparisons.

Table 5. Summary of ANOVA statistics for net energy yield among various ant-types.

Source	<i>DF</i>	Sum of Squares	Mean Square	<i>F</i>
Model	5	0.1098	0.0220	3.3763
Error	94	0.6113	0.00650	$P < 0.0075$

C Total	99	0.7210	0.00728
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Table 6. Mean net energetic yield for various ant-types.

Ant-Type (<i>n</i>)	Mean*	Std Error
Large ant, large leaf (23)	0.1359 a	0.01681
Large ant, small leaf (11)	0.0750 ab	0.02431
Medium ant, large leaf (15)	0.1428 a	0.02082
Medium ant, small leaf (19)	0.1186 ab	0.01850
Small ant, large leaf (12)	0.1475 a	0.02328
Small ant, small leaf (20)	0.0611 b	0.01803

*Means followed by the same letter are not significantly different at $\alpha = .05$ based upon the Tukey-Kramer HSD Test of Multiple Comparisons.

A comparison of biodiversity in a core and edge habitat of La Selva

Category: Independent Research
Participants: Heather Fowler and Jesse Greenston
Site: La Selva
Key Words: Biodiversity, core habitat, edge effects, insect diversity, plant diversity

Introduction

Nature reserves are becoming an increasingly important factor in preserving the biodiversity of tropical rainforests. One critical consideration of nature reserve design is how large a reserve should be to most effectively conserve biodiversity (Ashton 1986). The most widely accepted model argues for large reserves in order to minimize “edge effects” (Meffe et al. 1997). Reserve edges, defined as the zones of influence that constitute the outer boundary of a habitat, are an inherent feature of any reserve that borders deforested area (Meffe et al. 1997). Most studies have found that the environmental changes caused by creating forest edge have detrimental and profound effects on the ecology of the resulting forest fragment. Due to increased amounts of sunlight, secondary vegetation growth is stimulated, causing resource competition with primary growth (Meefe et al. 1997). The death of many highly endemic primary plants, because they cannot compete with the rapid growth rates of secondary plants, makes room for increased generalist plant growth. Insect herbivory increases but as specialized plants die, so do their associated specialized insects, creating a generalist insect population as well (Ronald Vargas pers. comm.). Thus loss of biodiversity is directly correlated with edge amount (Terborgh 1992).

The core of a reserve is the interior area that is not influenced by “edge effects”. Because it is buffered from these by the reserve edge and intervening forest, the core is predicted to have greater species diversity, high endemism and a decreased mortality rate (Meefe et al. 1997). Specialized plant species are able to dominate core habitats because resource partitioning allows a diversity of plant species to reside in the same area. The fierce resource competition common in edge habitat does not occur in the core.

Edge and core habitats differ, as well, in the nature and level of disturbance that they experience. Natural and anthropogenic disturbances affect edge habitats while only natural disturbances can affect a core habitat. The edge is also characterized as an area of high disturbance frequency and intensity. Models based on the intermediate disturbance hypothesis predict that the maximum amount of diversity is maintained in areas of moderate, discrete natural disturbances. Edge habitats, however, are influenced by long-term, anthropogenic disturbances. In addition, the intermediate disturbance hypothesis cannot be applied to edge habitats because it rests on the premise that a minimum dynamic area is maintained to allow an area to recover from disturbance. Edge habitats do not maintain this minimum area and thus are not resilient to change.

This study seeks to quantify the effects of anthropogenic disturbance upon the biodiversity of a reserve. Whereas natural disturbance occurs throughout a reserve, regardless of location, anthropogenic disturbances are concentrated at the edge and are nonexistent in the core habitat. Given this contrast in anthropogenic disturbance regimes we sought to find out if there were differences in biodiversity between an edge habitat and a core habitat of La Selva.

To compare biodiversity we sampled insect and plant populations in both habitats. We predicted that plant and insect diversity would be greater in the core habitat. We also predicted that there would be more overall plant and insect individuals in the edge habitat. Soil composition, however, can also affect biodiversity, especially plant diversity (Miguel Cifuentes pers. comm.). A decrease in soil nutrients or increase in soil pH can result in a loss of plant diversity and subsequent loss of insect diversity (Vandermeer et al. 1995). Thus, in order to properly interpret our biodiversity comparison we determine the basic soil properties for the selected habitats. We predicted that there would be fewer nutrients in edge soil because increased wind, rainfall, and sunlight at forest edges should result in increased soil erosion and nutrient leaching (Vandermeer et al. 1995). We also predicted that the soil pH would be higher in the edge than in the core habitat for the same reasons.

Methods

This study took place from November 16 to December 4, 1998 at La Selva Biological Field Station in Sarapiquí, Costa Rica. The core site was calculated by the Geographical Information (GIS)

computer system to be at 700 to 1100 m on the Camino Central trail. The edge site, located 100 m North of the Rio Puerto Viejo and East of the family houses of the station, is twenty year old reforested cattle pasture.

To determine plant diversity, three parallel 2 m by 50 m transects were performed at each site and all trees with a dbh (diameter at breast height) greater than 2.5 cm were counted, sampled for identification, and identified. Orlando Vargas, Assistant Director of Natural Science, identified all plants. Insect diversity was sampled by collecting leaf litter and collecting drowned insects from white and yellow pit fall traps of soapy water placed in the transects. Insects were extracted from leaf litter using the Burlese funnel. Ronald Vargas, a parataxonomist with the ALAS project, identified insects.

We collected two soil samples from each site. Each sample consisted of soil from ground level to 20 cm deep. Plant diversity at the species level and insect diversity at the family level was calculated using the Shannon-Weiner diversity index and the Shannon-Weiner *t*-test correlate to compare the edge and core habitats. We tested the soil samples for ammonium and phosphorous content using calometric determination procedures (Anderson et al. 1989) and measured soil pH using the Corning pH meter 245.

Results

A greater number of plant individuals were found in the edge site (Table 1). Plant diversity at the level of species was greater in the core site than in the edge site ($H'_{\text{core}} = 1.30$, $H'_{\text{edge}} = .909$, $t = -12.43$, d.f. = , $P < .001$). Diversity at the level of family was also found to be

higher in core plants ($H'_{\text{core}} = 1.11$, $H'_{\text{edge}} = .686$). Conversely, the edge habitat exhibited higher insect diversity than the core ($H'_{\text{edge(family)}} = .909$, $H'_{\text{core(family)}} = .431$, $H'_{\text{edge(order)}} = .559$, $H'_{\text{core(order)}} = .291$, $t = 9.99$, d.f. = , $P < .001$) but more insect individuals were found in the core (Table 2). The insect results, however, are not representative of diversity levels because 426 individuals identified to be of the army ant family *formicidae* were collected in a pit fall trap in the core habitat. Because ant colonies have one reproductive individual they can be counted as a single individual. When these are excluded, insect diversity is greater in the core habitat ($H'_{\text{core(family)}} = 1.21$, $H'_{\text{edge(family)}} = .909$, $H'_{\text{core(order)}} = .591$, $H'_{\text{edge(order)}} = .559$, $t = 7.57$, d.f. = , $P < .001$) and the number of insect individuals is greater in the edge habitat.

No differences were found in phosphorous ($t = .800$, d.f. = 7, $P = .4$) or ammonium ($t = .309$, d.f. = 7, $P = .7$) concentration between the soil samples. Measurements of pH also did not differ ($t = .213$, d.f. = 3, $P = .7$).

Discussion

The intermediate disturbance hypothesis assumes a disturbance regime based on natural disturbances. In comparison, “edge effects” occur as a result of anthropogenic disturbances. The core of a reserve does not experience anthropogenic disturbances while the edge habitat is characterized by anthropogenic disturbance. Thus, in comparing the biodiversity of core and edge habitats we sought to quantify the effects of anthropogenic disturbance upon the biodiversity of a reserve. Our data presents evidence that the number of plant and insect

individuals and their diversity levels differ in edge and core habitats. Soil does not appear to be affecting diversity levels, suggesting that “edge effects” are maintaining these biodiversity patterns. The decreased diversity of plants and insects in the edge habitat is most likely the result of changed environmental conditions at the reserve edge. Generalist species dominate the reserve edge because they must grow and reproduce rapidly despite environmental stress created through increased sun, wind, and rain exposure as well as increased herbivory rates and possible agrochemical exposure. Most edge insects are generalized herbivores because they must feed despite a high rate of tree mortality and the constant regrowth of different species. Specialization occurs in core habitats because fierce competition for resources is not a priority and the long life of plant species allows co-adaptive strategies between plants and insects time to evolve.

Plant individual and insect individual counts were higher, as predicted, in the edge habitat. Whereas core habitat plants are more liberally spaced to allow for resource sharing, edge plants compete for resources resulting in an increase in plant species turnover rate. A high turnover rate means that light gaps are created often. Increased exposure to sunlight stimulates growth explosions that yield the characteristic high plant density of the edge habitat. This dense plant growth is an ideal habitat for a generalist herbivore, and the constant regrowth of plants means that their food supply is never-ending. Thus, it is no surprise that the edge exhibited greater amounts of herbivory than the core habitat (Fowler et al. unpublished data). Edge habitats might also have higher insect

populations because they are more accessible to herbivores from nearby agricultural plots. The majority of these insects are also generalists so they are not limited by the abundance of a specific plant species (Ronald Vargas pers. comm.).

Our soil results showed no significant difference in soil composition between the sites, implying that soil is not a determining factor in the biodiversity differences of the sites. However, a more thorough soil analysis including tests for potassium, nitrate, and carbon levels might reveal differences. There are many factors besides erosion and leaching that can affect soil nutrients. The trees that inhabit an area can influence nutrient levels based on their absorption strategies. Nitrogen fixing plants, for example, drain the soil of great amounts of nitrogen. Thus, in order to determine if soil composition influences or changes with habitat biodiversity, a more complete study involving detailed analysis of soil and plant absorption strategies is necessary.

There are many future tests that could be performed to further understand the implications "edge effects". Replicates of our study using multiple edge habitats with detailed information of neighboring land use could identify the most influential factors leading to "edge effects". Biodiversity analyses of avian, mammalian, and amphibian species would provide a more complete picture of diversity differences in edge and core habitats and possibly illustrate trends in how edge effects influence the biota of an environment. It would also be interesting to study different plots from the edge to the core to see if a

gradient of biodiversity exists and how this gradient varies based on what type of diversity is studied.

In conclusion, "edge effects" must be considered in order to maximize the benefits accrued in developing nature reserves. It is apparent that nature reserve design is becoming the most important factor in preserving biodiversity. Understanding what the best design is for a given reserve must become a priority of research in conservation biology.

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Table 1: A comparison of number and type of plants in a core and edge habitat at the La Selva biological reserve in Costa Rica. The total number of individuals found in each family is shown in bold.

Family	Core	Edge
- Genus and species		
Annonaceae	2	0
- <i>Guatteria diospyroides</i>	2	0
Apocynaceae	1	0
- <i>Rauvolfia purpurascens</i>	1	0
Aracaceae	2	0
- <i>Asterogyne martiana</i>	1	0
- <i>Socratea exorrhiza</i>	1	0
Araliaceae	1	0
- <i>Deudropanax stenodontus</i>	1	0
Arecaceae	17	0
- <i>Welfia regia</i>	17	0
Bombaceae	1	0
- <i>Quararibea pumila</i>	1	0
Burseraceae	0	5
- <i>Protium pittierii</i>	0	5
Capparidaceae	0	1
- <i>Capparis pittierii</i>	0	1
Caricaceae	1	0
- <i>Jacaratia dolichaula</i>	1	0
Chrysobalaceae	1	0
- <i>Hirtella lemsii</i>	1	0
Lecythicaeae	1	1
- <i>Lecythis ampla</i>	1	1
Melastomaceae	2	0
- <i>Henriettca tuberculosa</i>	1	0
- <i>Micomia gracilis</i>	1	0
Meliaceae	2	4
- <i>Carapa nicaraguensis</i>	1	4
- <i>Guarea rophalocarpa</i>	1	0
Mimosaceae	1	42
- <i>Balizia elegans</i>	0	7
- <i>Pentaclethra macroloba</i>	1	35
Monimiaceae	1	0
- <i>Siparuna grandifolia</i>	1	0
Myristicaceae	1	0
- <i>Otoba norogranatensis</i>	1	0

<i>Papilionaceae</i>	2	49
- <i>Diptoryx panamensis</i>	0	29
- <i>Dussia macroprophyllata</i>	1	0
- <i>Louhocarpus oliganthus</i>	1	0
- <i>Pterocarpus rohrii</i>	0	20
<i>Piperaceae</i>	7	0
- <i>Piper coloneuse</i>	1	0
- <i>Piper pseudubombratum</i>	5	0
- <i>Piper (sp. Unknown)</i>	1	0
<i>Rhamnaceae</i>	6	0
- <i>Columbrina spinosa</i>	6	0
<i>Rubiaceae</i>	3	36
- <i>Psychotria elata</i>	1	0
- <i>Psychotria panamensis</i>	1	0
- <i>Psychotria (sp. Unknown)</i>	1	0
- <i>Simira maxonii</i>	0	36
<i>Sapindaceae</i>	1	0
- <i>Upania guatemalensis</i>	1	0
<i>Sapotaceae</i>	1	0
- <i>Pouteria (sp. Unknown)</i>	1	0
<i>Steruliaceae</i>	1	0
- <i>Theobroma simiarum</i>	1	0
<i>Tiliaceae</i>	2	0
- <i>Gochalsia meiantha</i>	2	0
Total	57	138

Table 2: A comparison of number and types of insects found in a core and edge habitat at the La Selva biological reserve in Costa Rica. The total number of individuals found in each order is shown in bold.

Order	Core	Edge
- Family		
Coleoptera	51	38
- <i>Carabidae</i>	2	4
- <i>Chrysomelidae</i>	1	0
- <i>Curculionidae</i>	3	3
- <i>Erotylidae</i>	2	5
- <i>Histeridae</i>	1	0
- <i>Hydrophilidae</i>	2	0
- <i>Pselaphidae</i>	4	4
- <i>Scolytidae</i>	8	7
- <i>Staphylinidae</i>	13	9
- <i>Trogosidae</i>	11	0
- Unknown	0	6
Dermaptera	2	0
- Unknown	2	0
Diptera	16	83
- <i>Dolichopodae</i>	4	0
- <i>Drosophilidae</i>	0	12
- <i>Micropezidae</i>	1	0
- <i>Phoridae</i>	2	26
- <i>Richardiida</i>	0	5
- Unknown	9	40
Hemiptera	4	18
- <i>Aradidae</i>	0	1
- <i>Thripidae</i>	2	10
- Unknown	2	7
Homoptera	2	0
- <i>Flatidae</i>	1	0
- <i>Lercopidae</i>	1	0
Hymenoptera	432	202
- <i>Bethylidae</i>	0	1
- <i>Braconidae</i>	0	3
- <i>Chalcidae</i>	5	2
- <i>Eupelmidae</i>	1	0
- <i>Evaniidae</i>	0	2
- <i>Formicidae</i>	426	175
- <i>Ichneumonidae</i>	0	1

- <i>Scoliidae</i>	0	17
Isoptera	1	0
- Unknown	1	0
Lepidoptera	3	2
- <i>Gracilaniidae</i>	0	1
- Unknown	3	1
Micro-hymenoptera	0	9
- <i>Chalcidoidea</i>	0	9
Molusco	1	0
- Unknown	1	0
Orthoptera	4	13
- <i>Gryllidae</i>	3	13
- <i>Tetrigidae</i>	1	0
<i>Psocoptera</i>	1	0
- Unknown	1	0
Total	517	365

Primary and secondary dispersal of *Dipteryx panamensis* fruits

Category: Independent Project
Participants: Jen Havlik and Sophia Kuo
Site: La Selva
Keywords: Agouti, *Dasyprocta punctata*, *Dipteryx panamensis*, primary seed dispersal, red-tailed squirrel, *Sciurus granatensis*, secondary seed dispersal

Introduction

Seed dispersal, the movement of seeds away from their parent plant, is a critical part of the plant life cycle (D. McClearn, pers.comm.). There are two phases of seed dispersal: primary and secondary. Primary dispersal is the movement of a seed away from the parent plant to a different surface (D. McClearn, pers.comm.). Secondary dispersal is the subsequent movement (following primary dispersal) of a seed in a horizontal or vertical direction (D. McClearn, pers.comm.). Plants rely on various dispersal agents, such as wind, birds, and mammals to move their seeds. The attraction of seed dispersal agents by fruiting plants is only one part of an evolved reproductive system (Bonaccorso et al. 1980).

We studied dispersal of *Dipteryx panamensis* (Fabaceae) fruits. In an experiment performed by Clark and Clark (1984), 83 of 101 *Dipteryx* fruits were within 9 m of the adult's bole. In the same study, none of the *Dipteryx* seedlings within 8 m of the parental bole survived (Clark and Clark 1984). This is consistent with the Janzen-Connell hypothesis, which states that "seeds that are most likely to survive to produce mature plants are those that are most isolated from established parental plants," (Thomas 1989). Thus, for *Dipteryx* seedlings to survive, it appears

that the fruits must be dispersed beyond the parental tree's seed shadow.

A variety birds and mammals are capable of dispersing *Dipteryx* fruits (Bonaccorso et al. 1980). In Panama, Neotropical red-tailed squirrels (*Sciurus granatensis*) have been observed carrying fruits a few meters beyond the parental crown (Bonaccorso et al. 1980). Agoutis (*Dasyprocta punctata*) on the other hand, were observed carrying *Dipteryx* fruits in excess of 50m from the parent tree (Bonaccorso et al. 1980). At the La Selva Biological Station in Costa Rica, little is known about mammalian seed dispersal, with the exception of bats and monkeys, both of which are arboreal (Levey et al. 1994). For this reason, we studied the effectiveness of two terrestrial mammals, squirrels and agoutis, as secondary dispersers of *Dipteryx panamensis* fruits. Based on the Bonaccorso et al. study (1980) in Panama, we hypothesized that agoutis would be more effective dispersal agents since they are capable of carrying the seeds further from the parent tree than squirrels.

Methods

We conducted our experiment at the La Selva Biological Station, located in the Sarapiquí region of Costa Rica. *Dipteryx panamensis* is a common emergent tree in this area, and is

characterized by smooth, salmon-colored bark, winged or subwinged rachis, and rather large, single-seeded, ellipsoidal fruit (Gentry 1993). *Dipteryx* fruit is 5-6 cm long by 2-3 cm wide, and weighs 16.0-26.3 g (Bonaccorso et al. 1980, personal observation). The endocarp is formed by a 4 mm thick, hard, woody shell enclosing the single seed (Bonaccorso et al. 1980). The outer covering, or exocarp, is formed by a thick pulp, similar to that of an almond fruit (Bonaccorso et al. 1980).

Sciurus granatensis (red-tailed squirrel) is both a predator and a disperser of *Dipteryx* seeds. Squirrels tend to carry the fruits toward the nearest tree, scrape off the pulpy exocarp at the narrow end of the seed, and then gnaw through the woody endocarp to expose the seed (Bonaccorso et al. 1980). After feeding, they frequently discard *Dipteryx* with a large part of the seed intact, usually including the embryo (Bonaccorso et al. 1980). Despite the damage, some of these *Dipteryx* seeds are still capable of germinating (Bonaccorso et al. 1980).

Dasyprocta punctata (agouti) are dependent on endocarps discarded by squirrels, and tend to prefer these damaged fruits (Bonaccorso et al. 1980). Using their long incisors, agoutis gnaw large holes through the endocarp and are often able to extract nearly all the seed via this hole. However, since agoutis scatterhoard (transport one piece of food at a time, or on rare occasions, a mouthful) and bury each fruit in its own hole away from the source (Hallwachs 1986), occasionally these *Dipteryx* seeds are forgotten or are only partially consumed (Bonaccorso et al. 1980), and they survive to germinate.

We examined primary and secondary dispersal of *Dipteryx* fruits. To

measure primary dispersal, we selected a fruiting *Dipteryx* tree located at approximately 250 m along the Camino Experimental Norte (CEN) trail. We measured two 30 m x 2 m transects and recorded the distance of each fruit in the transect from the bole of the tree on 22 November 1998. To measure secondary dispersal we used 4 rectangular "nut boxes" (patented by D. McClearn). Each nut box was made of clear plastic with a sliding lid, and each had 2 rows of 6 compartments, for a total of 12 compartments per box. We placed spools of thread in each of the compartments, and attached 48 fresh *Dipteryx* fruits to the ends of each of the threads using Super Bonder Extra glue (Loctite de Costa Rica, Centro Colón, Costa Rica). Apparently, the glue has no effect on the palatability of *Dipteryx* fruit (D. McClearn, pers.comm.). There were small grooves at the top of each compartment that allowed the thread to pass freely from the inside of the box to the outside once the fruit was moved.

On 22 November 1998 we placed two nut boxes 7 m away from the bole of a non-fruiting *Dipteryx* tree located approximately 100 m along the Camino Experimental Sur (CES) trail. Each of the 12 nuts from the two boxes were placed (with string attached) approximately 30 cm from their respective compartments. The other two boxes were placed 7 m away from the bole of the tree on the CEN trail where we measured primary dispersal. These fruits were also placed approximately 30 cm from their respective compartments. All of the boxes were covered with leaves to make them less conspicuous. Due to lack of activity at the CES tree, we moved these two boxes to the CEN site on 30 November 1998. The fruits from these

boxes were replaced with fresh fruits before being moved to the CEN site.

We examined the fruits each morning from 23 November to 5 December 1998. The following observations were recorded: distance from fruit to nut box, total distance traveled by fruit, viability (eaten / not eaten), status (buried / above ground), disperser (determined by characteristic bite marks to be either squirrel, agouti, or unknown), and number of days at the site before dispersal.

Results

We counted a total of 44 *Dipteryx* fruits within the two transects designed to measure primary dispersal. All of the fruits collected were found within the first 16 m of the transect, with the majority of them falling within the first 7 m (Figure 1). The mean distance of the fruits from the bole of the tree was 6.17 m (SD = 3.75).

All of the fruits from the nut boxes that had been visited were still viable – we observed bite marks from squirrels and agoutis that only penetrated part of the exocarp (Table 1). There was no damage to the seeds of these fruits. Of the four fruits that were removed, two traveled further than their final location from the tree, i.e. these fruits had been carried away from the nut box, and then were carried back toward the box before they were dropped (Table 1). Half of the fruits that had been visited were not moved at all (Table 1). Five out of eight of the fruits were disturbed within two days of being out at the site (Table 1). As seeds got older, they became moldy and soft, and were not disturbed by any animals to our knowledge.

Discussion

Our findings for the primary dispersal of *Dipteryx panamensis* seeds were consistent with the observations made by Clark and Clark (1984), in that the majority of the seeds fell within the area of the parental crown. For a tree with large seeds (such as *D. panamensis*) to be reproductively successful, it must be able to attract dispersal agents and avoid seed predators (Bonaccorso et al. 1980). In Panama, *Dipteryx panamensis* is visited by various mammals, including squirrels and agoutis, which act as both seed predators and dispersers (Bonaccorso et al. 1980). At the La Selva Biological Station in Costa Rica, we found that squirrels and agoutis were neither helpful nor harmful to the reproductive success of the *Dipteryx* tree we studied. We expected to see *Dipteryx* fruits gnawed on and dispersed away from the parent tree, particularly by agoutis, but we did not observe this.

A possible explanation for squirrel and agouti dispersal ineffectiveness could be the quality of the *Dipteryx* fruits. Hallwachs (1986) discovered that the distance a guapinol (*Hymenaea courbaril*) pod is carried and the rate at which pods are removed are strongly influenced by the condition of a pod's fruit pulp. This could also be the case for *Dipteryx panamensis* fruits. We observed that as fruits aged, they became moldy and soft. Perhaps our *Dipteryx* tree is producing a "bad" fruit crop this season, which the mammals find unappealing. Hallwachs (1986) was "certain that agoutis can recognize a [guapinol] pod with good pulp by its odor; usually they gnaw open the good fruits first." Our data suggest that the mammals approached the fresher fruits, tasted them at the spot or after carrying them briefly, and

then discarded them, possibly because they found the fruit to be “low quality.”

Based on the lack of damage done to the seeds and their small movement away from the seed shadow of the tree, it appears that neither the plant nor the mammals benefited from the fruiting that occurred. In terms of energy, the costs for both plant and animal appear to be high. The *Dipteryx* tree spent energy making the fruits, but none of them that we observed were dispersed past the crown. The squirrels and agoutis spent energy searching for the fruits, but did not consume them. Over time, this energy loss could become detrimental to both the tree and the mammals. On the other hand, we studied the effects of secondary dispersal at only one tree, which may not be representative of the entire *Dipteryx panamensis* population found at La Selva. Moreover, it is known that animals other than agoutis and squirrels act as dispersers of *Dipteryx* fruits. In their studies in Panama, Bonaccorso et al. (1980) suspected that spiny rats (*Proechimys semispinosus*) feed on *Dipteryx* fruits in a manner similar to squirrels, and act as occasional dispersal agents. In the same study, a fruit bat (*Artibeus lituratus*) was observed carrying *Dipteryx* fruits away from the parent tree. Both of these animals are found at La Selva (Levey 1994), and could be involved in *Dipteryx* fruit dispersal.

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Table 1. Observations of *Dipteryx panamensis* fruits in the La Selva Biological Station, Costa Rica. Dispersers were identified by tooth marks left on the fruits.

Seed #	Distance from fruit to nut box (m)	Total distance traveled by fruit (m)	Viability	Status	Disperser	# of days at site before dispersal
1	1.36	9.24	not eaten	above ground	squirrel	1
2	0.42	7.88	not eaten	above ground	squirrel	1
3	0.06	0.06	not eaten	above ground	agouti	4
4	0.28	0.28	not eaten	above ground	unknown	8
5	0	0	not eaten	above ground	agouti	10
6	0	0	not eaten	above ground	agouti	2
7	0	0	not eaten	above ground	squirrel	2
8	0	0	not eaten	above ground	squirrel	2

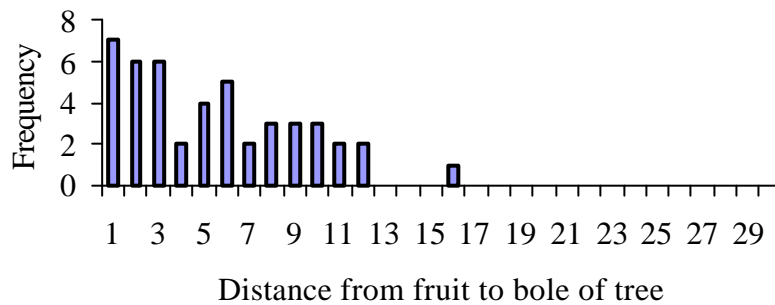


Figure 1. Primary dispersal of *Dipteryx panamensis* fruits from the bole of the parental tree, located at 250m along the CEN trail in the La Selva Biological Station, Costa Rica.

Kleptoparasitism in *Nephila clavipes*

Category: Independent Project
Participants: Sarah Huber
Site: La Selva
Key words: Araneidae, kleptoparasitism, *Nephila clavipes*, spiders

Introduction

Nephila clavipes (Araneidae) is the largest orb-weaving spider in the Neotropics (Lubin 1983). The species is most often found in secondary growth or clearings, although webs can be found in primary forest under story (pers. obs.). *N. clavipes* may build solitary webs or webs in aggregations containing several conspecifics (Lubin 1983). When prey items become entangled in the web, the spider immobilizes them by biting, removes them from the web, and returns to the center to feed (Lubin 1983). When the spider finishes feeding, it leaves partially digested prey items in the web. This behavior may attract a host of other organisms that feed on the leftovers or other small insects caught in the web. Vollrath (1979) found that kleptoparasites employed multiple tactics to steal or attack prey caught in *N. clavipes* webs, including feeding on partially digested insects. Personal observation suggests that webs built in open habitats and primary forest under story contain large amounts of kleptoparasites. *N. clavipes* webs are often abandoned by the host, a phenomenon that appears to be correlated with food availability and kleptoparasite density (Rypstra 1981).

This study sought to understand the rates of kleptoparasite colonization and web abandonment in open habitats and primary forest under story. Webs were observed in both types of habitats, and

kleptoparasites were examined. If web abandonment is correlated with kleptoparasite density, then I would expect to see abandonment in webs with higher numbers of parasites.

Methods

This study was conducted at La Selva Biological Station in Puerto Viejo de Sarapiquí, Costa Rica (rainfall 4,000 mm/year; elevation 35-150m). Kleptoparasites were monitored on 18 webs of *N. clavipes*: 8 webs were located in open, grassy areas, and 10 webs were located in the under story of primary forest. Each web was observed for 5 or 6 consecutive days between 30 November 1998 and 6 December 1998. Spider length and web dimensions were measured by placing a ruler next to the web or spider. Neither the web nor spider was disturbed during measurements. Web length and width were used to calculate web area according to the formula,

$$\text{Area} = \pi [(\text{length} + \text{width})/4]^2.$$

The day of abandonment or destruction was noted for each web. The number of kleptoparasites was recorded for each day, and all kleptoparasites were collected on day 5 or 6. Rates of colonization were determined by averaging the change in number of parasites between two consecutive days for the entire

observation period. For abandoned webs, colonization rates were determined both before and after the spider deserted the web. Kleptoparasite load was calculated as the number of kleptoparasites per cm². T-tests were conducted for comparisons between open and forest habitats and colonization rates. Chi-square analyses were used to compare the incidents of web abandonment and destruction in both habitats. Statistical analyses were done using JMP Statistical Analysis Package (SAS 1997).

Kleptoparasites collected from the webs were sorted by morphospecies, and species diversity of each habitat was calculated according to the Shannon-Wiener Index (H'). Evenness (J) was calculated and t-tests were conducted according to methods in Magurran (1988). Similarity was calculated according to the formula,

$$S = \frac{2C}{A + B},$$

where A is the number of morphospecies in the open habitat, B is the number of morphospecies in the forest habitat, and C is the number of morphospecies common to both habitats.

Results

There was no significant difference in the means for spider size, web area, number of kleptoparasites, and kleptoparasite load between open and forest habitat (Table 1). There was no correlation between web area and spider size ($r = 0.483$, $P = 0.0581$) or web area and number of kleptoparasites ($r = 0.5164$, $P = 0.0406$).

Figure 1 shows the number of kleptoparasites recorded for intact, un-abandoned webs in the open habitat. The number of parasites on intact, un-abandoned webs in primary forest under story are shown in Figure 2. The open habitat had a significantly lower rate of colonization in un-abandoned webs than the forest habitat and actually showed a negative trend (Table 1).

There were 3 abandoned webs in the open habitat and 1 abandoned web in the forest. There was, however, no significant difference in the incidence of web abandonment between habitats ($\chi^2 = 1.983$, $df = 1$, $P = 0.1591$). The web abandoned in the forest had a higher maximum number of kleptoparasites prior to abandonment than those found in the open habitat (Figure 3). Once the spider abandoned the web, the forest habitat had a more rapid rate of decolonization than the open area (Table 1). However, there was only one web in the forest that was abandoned, accounting for the large difference between the rates of colonization. There was no significant difference between colonization rates before and after abandonment in the open habitat ($t = -0.460$, $df = 7$, $P = 0.6593$, Table 1). Independent of habitat, there was a significant difference between the mean kleptoparasite load for abandoned (0.0129 ± 0.0045 individuals) and un-abandoned (0.00619 ± 0.00367 individuals) webs ($t = -2.380$, $df = 14$, $P = 0.0321$). Abandoned webs had a higher number of kleptoparasites per unit area than the un-abandoned webs.

In addition to the webs included in the previous analysis, there were 3 destroyed webs in the forest habitat and 1 in the open habitat; however, there was no

significant difference in the frequency of web destruction between habitats ($\chi^2 = 0.824$, $df = 1$, $P = 0.3641$). No kleptoparasites were found on the remaining web fragments following destruction.

There were 15 kleptoparasite morphospecies found in the open habitat and 11 found in the primary forest under story ($S = 0.462$, Figure 4). There was no significant difference in diversity between habitats ($H'_{\text{open}} = 1.06$, $H'_{\text{forest}} = 0.7295$, $t = 0.0959$, $df = 71$, $P > 0.05$). The evenness was higher in the open habitat than forest ($J_{\text{open}} = 0.90$, $J_{\text{forest}} = 0.70$).

Discussion

There were no habitat differences in spider size, web area, or incidents of web abandonment or destruction. Nor was web area indicative of either spider size or kleptoparasite load. Therefore, habitat structure and composition seem to have no effect on spider or web characteristics. However, this study did find three interesting phenomenon associated with kleptoparasitism of *Nephila clavipes*, regarding colonization rates, kleptoparasite loads and abandonment, and morphospecies distribution.

Colonization was lower in the open habitat than the primary forest under story. However, there was no difference in the mean number of kleptoparasites found in the two habitats (Table 1). The average number of parasites on webs in the open habitat were higher than those in the primary forest under story on day 1, but by day 5 and 6 the open habitat had a lower number of kleptoparasites than the forest. Thus, while the colonization trend was in different directions for each habitat, the mean number of parasites on the webs

were equal. Since webs were chosen at random and at varying stages of colonization, this trend may reflect a chance event. Future experiments measuring colonization rates should observe webs at similar stages of colonization (e.g. newly constructed webs).

Abandonment was higher in webs that had greater kleptoparasite loads. My data are consistent with those of Rypstra (1981) who found higher numbers of kleptoparasites on webs abandoned by spiders than webs not abandoned by spiders. This suggests that kleptoparasite load might be a possible reason for web abandonment in *N. clavipes*. Food availability may also affect abandonment. Beamer (unpublished data) studied the effects of food availability on site fidelity in *N. clavipes* and found that feeding was independent of relocation rates. Future experiments examining the interactions of kleptoparasites and food availability would be vital to understanding this system. Kleptoparasites that steal food from the host may deprive it from food sources and cause desertion. Kleptoparasites may make the web more visible to flying insects, decreasing the number of large insects that get caught in the web. *N. clavipes*, based on its relative size to that of the kleptoparasites, probably has a great food requirement. This may explain the persistence of kleptoparasites after web abandonment. In the open habitat there was no difference between colonization rates before and after abandonment. Kleptoparasites may continue feeding on small insects that are caught in the web, insects that are too small for the *N. clavipes* to consume. I also observed kleptoparasites preying on each other. If food availability is the impetus for

kleptoparasite colonization, then as long as food is available kleptoparasites should persist on the web.

Very little is understood about the specific interactions of kleptoparasite morphospecies. While diversity did not differ between habitats, analysis of morphospecies showed only 30.0 % overlap between the open habitat and primary forest under story. This may be a result of source populations or proximity to other webs. Perhaps specific morphospecies have physiological, life cycle, or micro habitat restrictions that limit their range. Therefore, only certain species act as sources in certain habitats. A second suggestion could involve colonization patterns. Perhaps colonization of one species depends on the existence of others. Predation by kleptoparasites on kleptoparasites was observed. Kleptoparasites that often fall prey to others may avoid webs that contain their predators. Similarly, predatory kleptoparasites may be more likely to colonize webs that contain spiders on which they can feed. Future studies examining colonization patterns could provide vital information on inter-specific kleptoparasite interactions.

Kleptoparasite colonization may also be related to niche partitioning. The two habitats had a relatively even distribution of kleptoparasites, and in the open habitat no one species dominated. Future studies looking at location of kleptoparasite morphospecies on the web and niche partitioning within the web would provide more information on potential competitive interactions between kleptoparasites. While morphospecies composition may be the result of some very interesting

processes, its effect on *N. clavipes* unknown at present.

Very little is known about the dynamic interactions between *N. clavipes* and its kleptoparasites. My data suggest that characteristics of the spider and its web seem to have little effect on kleptoparasites. However, kleptoparasite interactions, combined with host prey availability, may cause abandonment in the *N. clavipes*. Even more interesting is the possible role of inter-specific interaction between kleptoparasites in determining colonization rates and food availability, factors greatly affecting the host's behavior. Future studies concentrating on the specific behavior of kleptoparasites would provide valuable information on *N. clavipes* web dynamics.

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Table 1 Mean values between webs of *N. clavipes* in open and primary forest under story habitats. Statistical analyses were performed using JMP 3.2 Statistical Analysis Package (SAS 1997).

	Open Habitat	Forest Habitat	<i>t</i>	df	<i>P</i>
Mean spider length (cm)	3.08 ± 1.32 (n = 7)	3.02 ± 0.95 (n = 10)	-0.12	15	0.906
Mean web area (cm ²)	931.22 ± 733.16 (n = 6)	1192.04 ± 317.52 (n = 10)	0.997	14	0.336
Mean number of kleptoparasites	7.29 ± 4.00 (n = 8)	8.40 ± 5.98 (n = 10)	0.450	16	0.659
Mean kleptoparasite load (# of kleptoparasites/cm ²)	0.0071 ± 0.0045 (n = 6)	0.0070 ± 0.0044 (n = 10)	-0.080	14	0.937
Mean rate of colonization before abandonment	-2.04 ± 4.47 (n = 6)	2.16 ± 2.54 (n = 10)	2.417	14	0.0299
Mean rate of colonization after abandonment	-1.00 ± 1.73 (n = 3)	-17.00 ± 0.00 (n = 1)	-8.000	2	0.0153

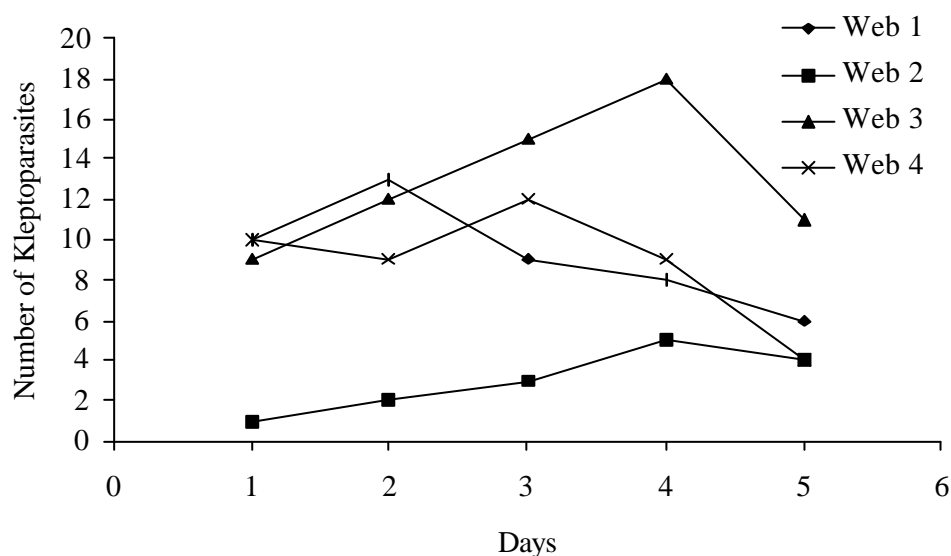


Figure 1. Intact, un-abandoned webs of *N. clavipes* were observed in open habitats. The number of kleptoparasites on the web was recorded for 5 consecutive days.

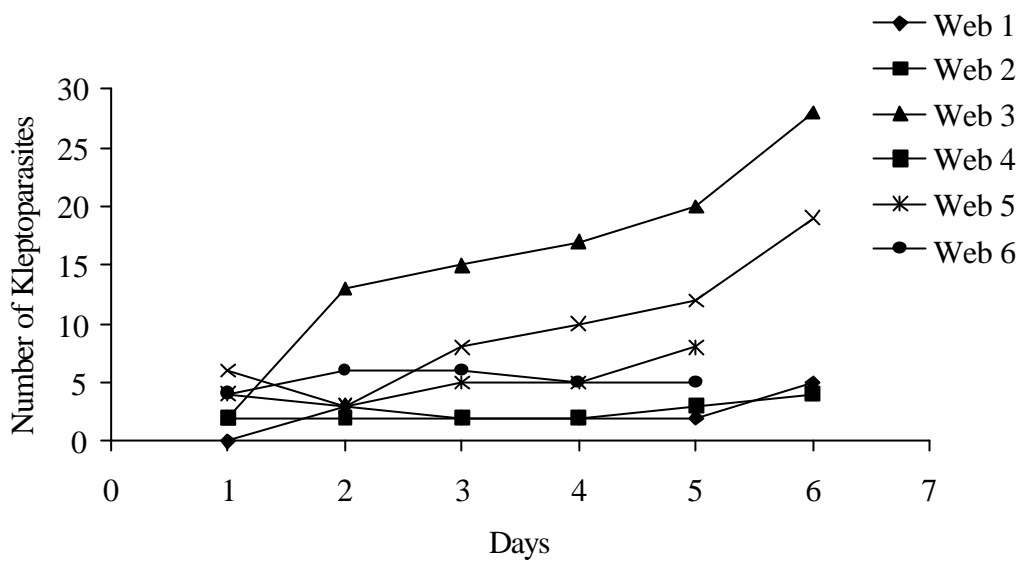


Figure 2. Intact, un-abandoned webs of *N. clavipes* were observed in primary forest under story. The number of kleptoparasites on the web was recorded for 5 or 6 consecutive days.

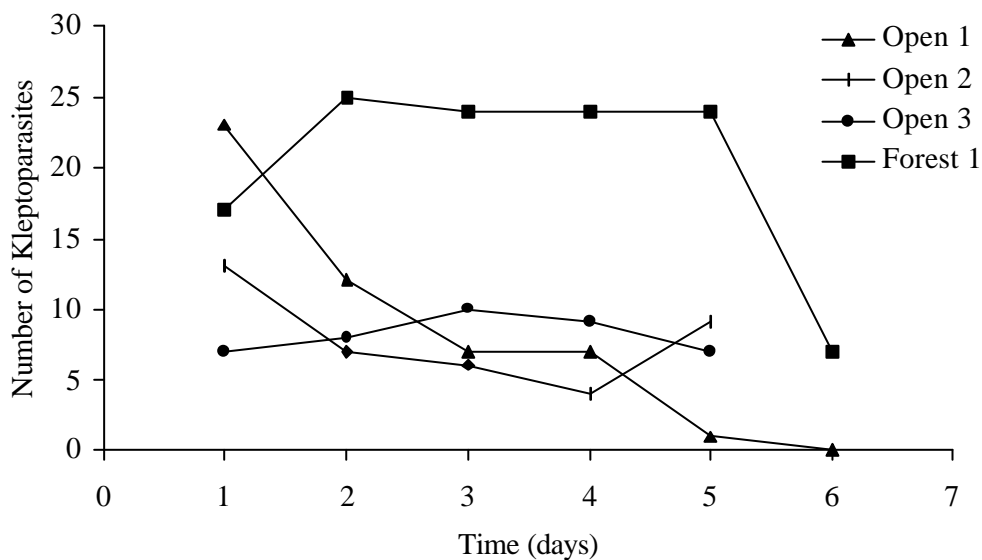


Figure 3. The number of kleptoparasites was recorded on webs of *N. clavipes* that were abandoned during the observation period. “Open” refers to webs in the open habitat. “Forest” refers to webs located in the primary forest under story. Open 1 was abandoned on day 3, Open 2 was abandoned on day 2, Open 3 was abandoned on day 1, and Forest 1 was abandoned on day 6.

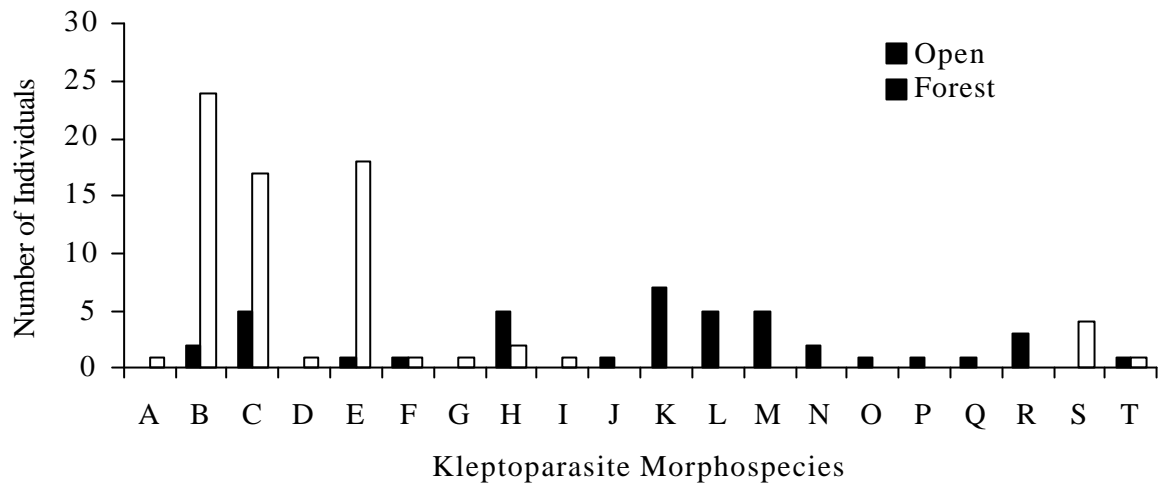


Figure 4. Number of kleptoparasite morphospecies found on webs of *N. clavipes*. Kleptoparasites were collected from all webs on day 5 or 6 of the study.

Differences in soil retention between monoculture and polyculture plots of *Hyeronima alchorneoides*

Category: Independent Project
Participants: Abigail C. Keys and Darren C. Miao
Site: La Selva
Keywords: Agroforestry, *Hyeronima alchorneoides*, nutrient retention, productivity, polyculture

Introduction

The conversion of tropical forests to agricultural systems of various kinds during the last few decades has caused widespread ecological problems, such as loss in soil fertility and genetic impoverishment (Schreckenberg and Hadley 1991). These actions have caused large spans of land in the tropics to become productively useless. The destruction of tropical forests and woodlands increased from 11.1 million ha / yr. in 1988 to 17 million ha / yr. in 1990, or approximately 1 % of the total estimated area of tropical forest area according to estimates by FAO (Schreckenberg and Hadley 1991). In response, there has been an overwhelming increase in the demand and frequency of reforestation efforts, secondary forest management, biomass production, and agroforestry in the humid tropics (Haggar and Ewel 1995). This has underscored the need to better understand how different tree species can be grown to maximize productivity and nutrient retention in nutrient poor soils.

The observation that mixtures of two plant species often have a higher productivity than either species grown alone has led to the idea that some species have the capacity to exploit resources unavailable to others (Haggar and Ewel 1994). Complementarity involving combinations of

crops and trees (polycultures) not only increase production but also conserve soil fertility (Haggar and Ewel 1997). Complementary use of resources in time occurs when plants have different temporal growth patterns or life spans, so that the periods of maximum demand for resources of the species are temporarily separated. It also occurs when different forms of a resource are used (nitrate vs. diatomic nitrogen), or when resources are used in different ratios (Haggar and Ewel 1997).

Although differences in productivity have been indicated, little attention has been given to polycultures and the differences they can make in soil nutrient retention. The use of polycultures is potentially important for agricultural and forestry systems, even though it is not usually associated with ecological economic sustainability (Haggar and Ewel 1994). If polycultures can make a difference in the maintenance of soil fertility, this method may increase the likelihood of maintaining productivity on nutrient poor soils.

This study investigates the differences in the retention of nutrients between polyculture and monoculture plots of *Hyeronima alchorneoides*. The null hypothesis is that soil samples taken from polyculture plots of *H. alchorneoides* will have a higher retention of nitrogen, ammonium, phosphorous, and organic carbon than

monoculture plots of *H. alchorneoides*. It is predicted that the polyculture plots will be able to retain a higher concentration of nutrients than the monoculture plots because of the complementarity of the species grown in the polyculture plots.

Methods

The experiment was carried out on the Huertos plots at La Selva Biological Station in the Atlantic lowlands of Costa Rica at an elevation of approximately 40 m. Mean annual rainfall and temperature are approximately 4 m and 24 ° C, respectively. The Huertos site is on an alluvial terrace with a deep, well drained, fertile soil classified as a mixed, isohyperthermic, possibly andic, fluventic, Dystrocept (Hagggar and Ewel 1994).

In 1991 Huertos project developers cleared an abandoned cocoa plantation, harvested the commercially valuable overstory trees, and burned the slash in order to set up monoculture and polyculture plots (Hagggar and Ewel 1997). These experimental plots were established after the manual clearing of the charred logs. The goal of the Huertos project was to assess the role of diversity and rotation frequency on sustainability.

Our study was conducted at the Huertos site where three different stands (60 x 40 m) of *H. alchorneoides* were subdivided into two plots; one plot (30 x 40 m) remained as a monoculture, and the other (30 x 40 m) was interplanted with the two monocots, *Euterpe oleracea* and *Heliconia imbricata*. The *Hyeronima* were planted in a triangular pattern with 2.0 m between each tree. Thus, each tree had available to it an area of 3.46 m², and the stand density was 2887 trees/ha for each plot (Hagggar and Ewel 1997). The

Euterpe were planted at the same time as the *Hyeronima* and were located between every other tree in every other row of trees. The *Heliconia* were planted between all the trees in the rows where *Euterpe* had not been planted.

Hyeronima alchorneoides, is a member of the Euphorbiaceae family native to Costa Rica, commercially valuable, and a fast growing tropical hardwood (Gentry 1993). *Euterpe oleracea* is a member of the Arecaceae family, multistemmed, tall (up to 20 m), and a pinnately leafed palm that grows in forests on alluvial soils (Gentry 1993). It is native to the lower Brazilian Amazon where its fruits and buds are harvested (Hagggar and Ewel 1997). *Heliconia imbricata* is a member of the Heliconiaceae family and is a native perennial herb common in the secondary forest around the site (Hagggar and Ewel 1997). It produces numerous basal shoots, each producing leaves that extend to 5 m or more in height (Gentry 1993).

Huertos chose these species because of their contrasting phenologies and physiognomic characteristics. For example, the roots of *H. alchorneoides*, *E. oleracea*, and *H. imbricata* are very different. *H. alchorneoides* and *E. oleracea* have a very high density of fine roots, where as *H. imbricata* have a low density of fine roots. *H. alchorneoides* and *H. imbricata* have shallow roots, where as *E. oleracea* have very deep roots. They also differ in their growth patterns. *H. alchorneoides* and *E. oleracea* have apical meristems which facilitate vertical growth, but *H. imbricata* has a basal meristem that facilitates increasing width while constraining vertical growth. *H. alchorneoides* and *H. imbricata* allocate more energy in building leaves, while *E.*

oleracea allocate more energy building roots. These differences may indicate a potential for different resource capture capabilities (Haggar and Ewel 1997).

Three core samples of soil at a depth of 30 cm were taken randomly from each monoculture and polyculture plot within the three *H. alchorneoides* stands. Analyses of the nutrient composition of nitrogen, ammonium, phosphorous, and organic carbon in the soil samples were conducted following the guidelines set in Tropical Soil Biology and Fertility: A Handbook of Methods (Anderson and Ingram 1989).

Leaf litter from two random 1 x 1 m plots within each monoculture and polyculture plot was collected to compare soil coverage between the plots. Wet weight was measured using an electronic scale, and six of the twelve collections were dried in a herbarium (a drying oven for organic matter). The six dried samples were then weighed using an electronic scale, and the percent water loss was calculated. The average water loss for the six dried samples was used to estimate the dry weight of the other six leaf litters. The percent of overstory canopy allowing the filtration of light was determined at each of the two leaf litter plots within the polyculture and monoculture plots using a spherical densometer. Light intensity was measured in LUX units at all coring sights at ground level using a light meter.

Above ground biomass was calculated using the formula $y = e^{[-3.3012 + 0.9439 \ln (D^2H)]}$ from Tropical Soil Biology and Fertility: A Handbook of Methods (Anderson and Ingram 1989). Within each monoculture and polyculture plot, a row of trees was selected, and the diameter breast height (DBH) of each tree

was measured. The tree height in the polyculture and monoculture plots was assumed to be identical for all *Hyeronima* individuals. Thus, the estimated height of all the *Hyeronima* individuals, 7.62 m, was used for calculating biomass.

Wilcoxin non-parametric tests were used to analyze all of the data except for tree DBH and biomass. Soil analyses for nitrate, ammonium, and phosphorous were done twice and denoted "a" and "b" to examine the accuracy of our methods. Wilcoxin sign rank paired t-tests were used to test for significant differences between soil analyses "a" and "b" for nitrate, ammonium, phosphorous, and organic carbon. Since the distribution of tree DBH and biomass was found to be normal, one-way anova tests were used to analyze these two sets of data.

Results

There was no significant difference between percentages of NO_3^- , $\text{NH}_4^+\text{-N}$, phosphorous, and organic carbon between soils from monoculture plots to polyculture plots (Table 1 – 4). Monoculture plots received significantly higher intensity levels of light than polyculture stands at ground level (Table 5). Polyculture stands had significantly less uncovered overstory than monoculture stands (Table 6) as well as significantly denser leaf litter (Table 7). Average *Hyeronima* diameter at breast height was not significantly different between monoculture stands and polyculture stands (Table 8) nor was *Hyeronima* above ground biomass significantly different (Table 9).

NO_3^- percentage levels obtained from soil analyses "a" and "b" were not significantly different from each other for monoculture stands ($T = -11.5$ and $P =$

0.203), although they were significantly different for polyculture stands ($T = -22.5$ and $P = -0.004$). However, both analyses yielded no significant differences in NO_3^- percentage levels between monoculture and polyculture stands ($P = 0.9248$ for test 1, $P = 0.9296$). NH_4^+ -N percentage levels obtained from analyses “a” and “b” were not significantly different from each other for both monoculture stands ($T = -5.500$ and $P = 0.570$) and polyculture stands ($T = -2.50$ and $P = 0.820$). Additionally, phosphorous percentage levels obtained from analyses “a” and “b” were not significantly different from each other for both monoculture stands ($T = 11.5$ and $P = 0.203$) and polyculture stands ($T = -3.50$ and $P = 0.734$).

Discussion

The results showed that there was no significant difference between nutrient concentrations of nitrogen, ammonium, phosphorous, and organic carbon between the soils from monoculture and polyculture plots. Although polyculture plots exhibiting complementarity in general have been shown to increase soil fertility and nutrient retention (Haggard and Ewel 1997), our study shows that this is a trend that can not be generalized among all polyculture plots. The species that are planted in a polyculture plot must have different temporal growth patterns, use different forms of resources, or use different ratios of resources to exhibit complementarity. If these differences were not significant enough between *Hyeronima*, *Heliconia*, and *Euterpe*, then this could explain why the polyculture plots did not retain more nutrients than the monoculture plots. However, if this was the case, we might expect to see lower productivity as a result of interspecific competition for the

same nutrients in the polyculture plots if these species have an overlap in the nutrients they exploit. Because productivity of *H. alchorneoides* and overall productivity were found to be the same between polyculture and monoculture plots (personal comment M.Cifuentes), something else must be occurring.

Our results showed significant differences in light intensity, uncovered overstory, and leaf litter between the monoculture and polyculture plots. These differences may account for a system of nutrient cycling that supports our results of no net difference in nutrient composition between the monoculture and polyculture plots. The light intensity and % uncovered overstory measured from ground level was significantly higher in monoculture than polyculture plots. If more light was able to penetrate the forest floor of the monoculture plots and there was a higher % of uncovered overstory, then the canopy of the monoculture plots must not be as dense as the polyculture plots. If this were true then more rainwater would have direct contact with the forest floor of the monoculture plots because of its less dense canopy. This could have lead to a greater rate of nutrient loss due to leaching of nutrients in the monoculture plots, especially since there was less leaf litter to act as a barrier from the rain in monoculture plots.

Although the canopy density and leaf litter of the polyculture plots appeared to be responsible for the increased retention of nutrients, the addition of *H. imbricata* and *E. oleracea* to the polyculture plots may have increased the uptake of nutrients from the soil. It is possible that the addition of *H. imbricata* and *E. oleracea* increased total above ground biomass, creating a more extensive root system, in the

polyculture plots. This extensive root system may facilitate the uptake of more nutrients due to the demand for more resources. Therefore, the nutrient uptake by the species in the polyculture plots may balance out the nutrient loss in the monoculture plots resulting in no net difference in concentration of nitrate, ammonium, phosphorous, and organic carbon between the monoculture and polyculture plots.

Although the polyculture plots did not serve to increase the fertility and nutrient retention of the soil, there still are benefits to this method of agriculture. The polyculture plots were able to maintain productivity with out depleting the soil of nutrients while yielding a crop that had a higher commercial value than the monoculture plots. This would benefit subsistence farmers who want high commercial value crops with low maintenance costs. So even though the polyculture method is not always ecologically beneficial, it has other advantages in not harming the environment, having a low maintenance cost, and by being economically beneficial.

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Table 1. Average nitrate percentage levels in soils of three monoculture plots of *Hyeronima alchorneoides* and in three polyculture plots of *Hyeronima alchorneoides*, *Euterpe oleracea*, and *Heliconia imbricata* at La Selva Biological Station, Costa Rica. “a” and “b” denote separate analysis tests. Nitrate was extracted with 2M potassium chloride.

	% NO ₃ ⁻ present in soil		Z - value	P - value	S
	Monoculture	Polyculture			
Plot 1 a.	12.7425	3.2599			
b.	11.4691	10.6284			
Plot 2 a.	5.0193	4.4256			
b.	13.5984	13.5984			
Plot 3 a.	0	2.1028			
b.	13.5926	13.5926			
Mean ± 1 SE	9.4037 ± 2.29643	8.1558 ± 2.2998	-0.040032	0.6889	36

Table 2. Average nitrogen percentage levels in soils of three monoculture plots of *Hyeronima alchorneoides* and in three polyculture plots of *Hyeronima alchorneoides*, *Euterpe oleracea*, and *Heliconia imbricata* at La Selva Biological Station, Costa Rica. “a” and “b” denote separate analysis tests. Nitrogen was extracted with 2M potassium chloride.

	% NH ₄ ⁺ - N present in soil		Z - value	P - value	S
	Monoculture	Polyculture			
Plot 1 a.	2.5462	2.3646			
b.	3.2081	3.6404			
Plot 2 a.	2.0039	2.1744			
b.	3.0189	1.2968			
Plot 3 a.	1.6184	2.0608			
b.	0.6679	2.5810			
Mean ± 1 SE	2.1772 ± 0.38844	2.3530 ± 0.31318	0.24019	0.8102	41

Table 3. Average organic carbon percentage levels in soils of three monoculture plots of *Hyeronima alchorneoides* and in three polyculture plots of *Hyeronima alchorneoides*, *Euterpe oleracea*, and *Heliconia imbricata* at La Selva Biological Station, Costa Rica. Organic carbon was extracted using a potassium dichromate and sulfuric acid digest.

	% organic C present in soil		Z - value	P - value	S
	Monoculture	Polyculture			
Plot 1	0.7221	0.8136			
Plot 2	0.3372	0.8119			
Plot 3	0.6194	0.2631			
Mean \pm 1 SE	0.5424 \pm 0.11515	0.7025 \pm 0.17352	0.63901	0.5228	34

Table 4. Average phosphorous percentage levels in soils of three monoculture plots of *Hyeronima alchorneoides* and in three polyculture plots of *Hyeronima alchorneoides*, *Euterpe oleracea*, and *Heliconia imbricata* at La Selva Biological Station, Costa Rica. “a” and “b” denote separate analysis tests. Phosphorous was extracted using 2M potassium chloride.

	% P present in soil		Z - value	P - value	S
	Monoculture	Polyculture			
Plot 1 a.	2.2807	0.4587			
b.	0.8285	4.0796			
Plot 2 a.	2.3373	1.6418			
b.	1.1406	5.0398			
Plot 3 a.	0.9456	1.9855			
b.	1.1699	0.1136			
Mean \pm 1 SE	1.6083 \pm 0.31025	2.062 \pm 0.81434	-0.08006	0.9362	38

Table 5. Average light intensity measured in LUX units at ground level in three monoculture plots of *Hyeronima alchorneoides* and in three polyculture plots of *Hyeronima alchorneoides*, *Euterpe oleracea*, and *Heliconia imbricata* at La Selva Biological Station, Costa Rica.

	Light Intensity (LUX)		Z - value	P - value	S
	Monoculture	Polyculture			
Plot 1	4357	890			
Plot 2	5380	1180			

Plot 3	6077	790			
Mean \pm 1 SE	5271 \pm 865	953 \pm 117	-3.416	0.0006	36

Table 6. Average percentages of uncovered overstory in three monoculture plots of *Hyeronima alchorneoides* and in three polyculture plots of *Hyeronima alchorneoides*, *Euterpe oleracea*, and *Heliconia imbricata* at La Selva Biological Station, Costa Rica.

	% Overstory Uncovered		Z - value	P - value	S
	Monoculture	Polyculture			
Plot 1	47.9	22.9			
Plot 2	48.4	21.3			
Plot 3	66.0	38.5			
Mean \pm 1 SE	54.1 \pm 5.95	27.6 \pm 5.48	-2.4863	0.0129	23

Table 7. Average mass of leaf litter gathered from two 1 m x 1 m transects in three monoculture plots of *Hyeronima alchorneoides* and in three polyculture plots of *Hyeronima alchorneoides*, *Euterpe oleracea*, and *Heliconia imbricata* at La Selva Biological Station, Costa Rica.

	Leaf Litter Mass (g)		Z - value	P - value	S
	Monoculture	Polyculture			
Plot 1	666.4	781.6			
Plot 2	531.0	853.4			
Plot 3	643.3	797.6			
Mean \pm 1 SE	613.5 \pm 41.82	810.9 \pm 21.76	2.48199	0.0131	55

Table 8. Average diameter breast height (DBH) in centimeters of *Hyeronima alchorneoides* trees in monoculture plots of *H. alchorneoides* and polyculture plots of *H. alchorneoides*, *Euterpe oleracea*, and *Heliconia imbricata* at La Selva Biological Station, Costa Rica.

	DBH (cm)		F - ratio	t	P	DF
	Monoculture	Polyculture				
Plot 1	18.9 (n = 8)	16.9 (n = 9)				
Plot 2	19.7 (n = 8)	18.7 (n = 9)				
Plot 3	17.3 (n = 8)	19.1 (n = 8)				
Mean \pm 1 SE	18.6 \pm 0.756	18.2 \pm 0.663	0.1863	0.432	0.6680	48

Table 9. Average above ground biomass in kilograms of *Hyeronima alchorneoides* trees in monoculture plots of *H. alchorneoides* and polyculture plots of *H. alchorneoides*, *Euterpe oleracea*, and *Heliconia imbricata* at La Selva Biological Station, Costa Rica. Biomass calculated using the wet tree biomass equation from Anderson and Ingram (1989). Height held constant at 7.62 m.

	Aboveground Biomass (kg)		<i>F</i> – ratio	<i>t</i>	<i>P</i>	DF
	Monoculture	Polyculture				
Plot 1	67.5 (<i>n</i> = 8)	53.9 (<i>n</i> = 9)				
Plot 2	70.1 (<i>n</i> = 8)	63.7 (<i>n</i> = 9)				
Plot 3	56.0 (<i>n</i> = 8)	67.6 (<i>n</i> = 8)				
Mean \pm 1 SE	64.5 \pm 4.33	61.8 \pm 4.08	0.2395	0.489	0.6268	48

The effectiveness of extrafloral nectaries in preventing herbivory of *Passiflora vitifolia*
(Passifloraceae)

Category: Independent Project
Participants: Gregory S. Mitchell and Nicole M. Donovan
Site: La Selva
Keywords: Ant-plant interactions, extrafloral nectaries, herbivory, *Passiflora vitifolia*

Introduction

Extrafloral nectaries are nectar secreting glands found outside of the flower and are a common feature of many tropical plants (Bentley 1977). They are hypothesized to protect the plant against herbivory in a two-step process. First, ants are attracted to the nectar in the extrafloral nectaries. Previous research has shown that increasing extrafloral nectar of plants attracts additional ants, and eliminating extrafloral nectar by covering the nectaries results in reduced ant visitation (Clarke 1991, McLennan 1990). Second, ants protect the plant from herbivory by killing or forcing potential herbivores off the plant (Wolin 1979).

We studied the effectiveness of the extrafloral nectaries of *Passiflora vitifolia* in attracting ants to prevent herbivory. The presence of ants on *Passiflora* plants has been shown to reduce herbivory, but in addition to ant presence, the number of ants present on a plant may also have an effect on the amount of herbivory sustained by that plant (Wolin 1979). More ants would be more likely to encounter herbivores and also would be more effective at deterring them. We investigated the connection between the ant recruitment by extrafloral nectaries and ants' role in preventing herbivory of *P. vitifolia*. We hypothesized that increasing the amount of extrafloral nectar made available by *P. vitifolia* would

increase ant recruitment and that increased ant recruitment would decrease the amount of herbivory sustained by the plants. We also hypothesized that decreasing the amount of extrafloral nectar available to ants would decrease ant recruitment, which would cause the plants to sustain more herbivory.

Methods

P. vitifolia ranges from Nicaragua to northern South America, and in Costa Rica is most common in low to middle elevation moist and wet forest (Smiley 1983). We selected *P. vitifolia* for our experiment because of its relative abundance and the presence of conspicuous extrafloral nectaries on the leaf margins and petioles. The principal herbivores of *P. vitifolia* are heliconiine butterfly larvae, heliconiine adults, and flea beetle (Alticini and Chrysomelidae) larvae and adults (Smiley 1983). We conducted our experiment from 11/18/98–12/3/98 at La Selva Biological Reserve, a lowland tropical wet forest in the Sarapaquí region of Costa Rica. Our sample consisted of 21 shoots of *P. vitifolia* located in 5 discrete areas in and surrounding the 3-4 and 4-5 year-old successional plots. We selected 7 shoots to be in either of two treatments or a control. In the first treatment, we shortened 2 microcentrifuge tubes to 1/2 of their original length and duct-taped them to the

stem of the shoot, at 1/3 and 2/3 of the length of the focal section. We then filled the tubes with a 2M sucrose solution. This treatment was designed to simulate increased extrafloral nectar production. In the second treatment we covered all visible extrafloral nectaries with nail polish to prevent ant access to the extrafloral nectar. This treatment was designed to simulate decreased or no extrafloral nectar production. The 7 control shoots were not manipulated. Because the extrafloral nectaries associated with older, fully expanded leaves often cease nectar production (Bently 1977), we included only the 7 youngest leaves of each shoot in our study in order to standardize the number of extrafloral nectaries (2 petiolar and 4 on the leaf margin per leaf) observed in each shoot. Before beginning each treatment, each investigator measured the initial area (cm^2) of herbivory present in the focal section of each shoot using a 100cm^2 grid, and the two independent readings were averaged for each leaf. One day elapsed between setting up the treatments and initial data collection on ant recruitment and non-ant visitors.

Ant recruitment was defined as the number of ants present in the focal section of each shoot and was measured at the beginning and again at the end of a 5-minute observation period. During the 5-minute period, each of us observed one shoot and recorded the number, location (i.e. stem, leaf, nectary, or fake nectary), and Order of the non-ant visitors to the focal section of the shoot. We collected data on each shoot 8 times in the morning and 8 times in the afternoon during the two week period. The sugar solution in the fake nectaries was replaced after each observation. At the end of each week, the area of herbivory in the

focal sections was again measured. The initial herbivory measurements were subtracted from the measurements at the end of each week to determine a difference in absolute herbivory over time. Herbivory measurements were compared across shoots under the assumption that a given herbivore will eat the same amount of leaf material regardless of the total leaf area available.

Because the number of ants and amount of herbivory was not normally distributed, we analyzed the data using non-parametric statistical tests. A Wilcoxon test was used to determine whether there was a difference in number of ants or herbivory between morning and afternoon. Kruskal-Wallis and Tukey-Kramer tests were used to determine whether there were significant differences in number of ants or amount of herbivory by treatment. A 2-way ANOVA was used to determine the effect of plant location, treatment, and the interaction of these two variables. Finally, regression analysis was performed to test the relationship between the average number of ants per shoot and amount of herbivory per shoot. All statistical analyses were performed using JMP statistical analysis program (SAS Institute Inc. 1996).

Results

We observed that ants and non-ants visited all of the fake nectaries (pers. obs.), suggesting that they successfully simulated increased extrafloral nectar production. The nail polish treatment appeared to be successful in simulating decreased or no extrafloral nectar production because we observed that neither ants nor non-ants visited the covered nectaries (pers. obs.). A 24-hour observation period after application of the

nail polish revealed no adverse effects of the nail polish to the leaves or ants.

A Wilcoxon test revealed no difference between the number of ants in the morning and in the afternoon ($Z = -0.57$, d.f. = 1, $P = 0.57$). Data from the morning and afternoon were therefore pooled for further analysis. A Kruskal-Wallis test revealed that the number of ants per shoot differed by treatment (Table 1). Specifically, the sugar and control shoots had a higher number of ants than the nail polish shoots, but themselves did not differ in number of ants, (Table 2). The amount of herbivory in each shoot also differed by treatment (Table 1). The control shoots had the greatest herbivory and the sugar shoots sustained the least herbivory. Differences in herbivory were significant between each treatment (Table 3). A 2-way ANOVA revealed that treatment, plant location, and the interaction of location and treatment each had a significant effect on ant recruitment, and the model accounts for 38 % of the variance in the data (Table 4). Likewise, treatment, plant location, and their interaction significantly affected the amount of herbivory on each shoot, and 41 % of the variance is included in the model (Table 5). A regression analysis reveals that the average number of ants per shoot and the amount of herbivory per shoot are unrelated ($R^2 = 6.0 \times 10^{-6}$, d.f. = 1, $P = 0.99$). The observational data reveal that shoots in the sugar treatment had more non-ant Hymenoptera visitors than either the control or the nail polish shoots, and that Diptera were the most common visitors to all shoots collectively (Table 6).

Discussion

Extrafloral nectaries are an important strategy employed by plants to

prevent herbivory. The results of our experiment suggest that extrafloral nectaries in *P. vitifolia* provide protection from herbivory, but the patterns observed in our results differed from our predictions. In particular, our results suggest that increasing extrafloral nectar production does not necessarily recruit more ants, and that ant recruitment and herbivory are unrelated. Furthermore, our results suggest that wasp visitation to the extrafloral nectaries, not ant recruitment, may be the plant's primary defense against herbivory.

We hypothesized that increasing the extrafloral nectar of *P. vitifolia* would increase ant recruitment, and that decreasing the amount of extrafloral nectar would decrease ant recruitment. The shoots with the nail polish treatment, simulating shoots with decreased or no extrafloral nectar production, recruited fewer ants than the control and sugar shoots, supporting our hypothesis. This is consistent Clarke's (1993) results obtained from covering the extrafloral nectaries of balsa plants. Unlike Clarke's (1993) results, however, the sugar treatment did not recruit more ants than the control shoots. This suggests that extrafloral nectar does attract ants, but that the plants were most likely already maximally recruiting ants. An increase in nectar availability, therefore, would have little effect on ant recruitment (McLennan 1990), but decreasing extrafloral nectar production or producing no extrafloral nectar significantly reduces ant recruitment to the plant. The sugar treatment shoots also may not have recruited additional ants because the sugar solution lacked amino acids found in the extrafloral nectaries of *P. vitifolia* (Smiley 1983). Our data also suggest that plant location had a significant effect on ant recruitment and that plants responded

differently to each treatment because of their location. Differences in proximity to ant colonies, ant species at a particular location, and microclimatic variation likely account for some of the variation by location. We observed two distinct ant species that did not coexist in the same location (pers. obs.). These species were not present in similar numbers and may have responded to changes in extrafloral nectar production differently. The ant-recruitment model accounted for less than half the variance in the data, suggesting that additional variables, most likely individual differences between plants, are also affecting ant recruitment. Differences in plant extrafloral nectar quality and other physiological or morphological variations could cause differential ant recruitment among individuals.

We also hypothesized that increasing the extrafloral nectar production of *P. vitifolia* would cause the plant to sustain less herbivory because of increased ant recruitment, and that decreasing extrafloral nectar production would cause the plant to sustain more herbivory. The results of our experiment are different than predicted; in particular, shoots with decreased extrafloral nectar (nail polish treatment) had less herbivory than controls. As with ant recruitment, the herbivory of each shoot was significantly affected by plant location and location affected plant responses to the treatments. Which herbivores are present in a particular location, risk of predation/parasitism (including by the species of ant present), and microclimatic variation between locations may change the susceptibility of a plant to herbivory. Individual differences between plants, including nutritional quality of leaves and strength of secondary

compounds, probably also account for variance in the herbivory model. These variations between locations and individuals altered the affect of treatment on plants. Additionally, herbivores may have avoided shoots with the nail polish treatment because of the taste or smell of the nail polish

Because number of ants (ant recruitment) and herbivory were unrelated, it appears that ant recruitment in this sample of *P. vitifolia* is not the plant's primary protection against herbivores. The assertion that ant presence improves a plant's level of protection from herbivory (Wolin 1979) does not appear to be true in our sample. Wolin (1979) also asserts, however, that ant species, in addition to number of ants, affects the level of herbivory and that the number of ants necessary for a certain level of protection varies between *Passiflora* species. Our sample of *P. vitifolia* may not have had an ant species that effectively prevents herbivory. During the 2-week experiment we never observed ants on any of the plants attacking herbivores (pers. obs.). Hespeneide (1985) hypothesized that visitors other than ants to extrafloral nectaries may be important in protecting plants against herbivory and that some plants may benefit more from parasitic Hymenoptera visiting the nectaries than from ant visitation. Predatory Hymenoptera indeed visit *Passiflora* and are attracted to extrafloral nectaries. In our experiment, *P. vitifolia* with the sugar treatment had many more non-ant Hymenoptera visitors such as wasps, and had much less herbivory, than the other shoots. Most of these non-ant Hymenoptera visitors were observed at the fake extrafloral nectaries (unpub. data). These Hymenoptera, not the ants as we predicted, may have been responsible for

protecting the shoots with the sugar treatment against herbivores and are likely the principal defenders of *P. vitifolia* in this site against herbivory.

Short term research at La Selva has been equivocal about the benefits of extrafloral nectaries (Marquis and Braker 1994). Further research on the effectiveness of extrafloral nectaries on preventing herbivory should be conducted over the long-term with genetically similar individuals in a homogenous habitat. This would control for as many variables as possible and would allow further investigation into the methods and effectiveness of extrafloral nectaries in preventing herbivory. Much of the research on extrafloral nectaries has assumed that ant recruitment by extrafloral nectaries is the method by which herbivory is prevented. Our research, however, strongly indicates that recruitment of non-ant Hymenoptera to extrafloral nectaries must be considered as an alternative strategy to prevent herbivory.

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Table 1. Mean number of ants and mean herbivory by treatment (n = 7 shoots per treatment) on *Passiflora vitifolia*, La Selva Biological Reserve, Costa Rica.

	Mean number of ants (SD)	Mean herbivory in cm ² (SD)
Sugar	8.90 (12.00)	2.51 (4.57)
Nail Polish	0.88 (1.74)	12.93 (15.43)
Control	8.32 (9.68)	19.34 (24.17)
Kruskal-Wallis Test	$X^2 = 191.75$ d.f. = 2 $p < 0.0001$	$X^2 = 234.41$ d.f. = 2 $p < 0.0001$

Table 2. Differences in number of ants by treatment (n = 7 shoots per treatment) on *Passiflora vitifolia*, La Selva Biological Reserve, Costa Rica.

** = significant at $\alpha = 0.05$

Treatment	Sugar (q*)	Nail Polish (q*)	Control (q*)
Sugar	---	---	---
Nail Polish	6.06**	---	---
Control	-1.33	5.45**	---

Table 3. Differences in herbivory by treatment (n = 7 shoots per treatment) on *Passiflora vitifolia*, La Selva Biological Reserve, Costa Rica.

** = significant at $\alpha = 0.05$

Treatment	Sugar (q*)	Nail Polish (q*)	Control (q*)
Sugar	---	---	---
Nail Polish	6.83**	---	---
Control	13.32**	2.79**	---

Table 4. Effect of treatment, plant location and treatment-location interaction on ant recruitment in *Passiflora vitifolia* (n = 21 shoots), La Selva Biological Reserve, Costa Rica. $R^2 = 0.38$

	d.f.	F	P
Treatment	2	47.83	< 0.0001
Location	3	26.32	< 0.0001
Treatment*Location	6	21.17	< 0.0001
Whole Model	11	36.64	< 0.0001

Table 5. Effect of treatment, plant location and treatment-location interaction on herbivory in *Passiflora vitifolia* (n = 21 shoots), La Selva Biological Reserve, Costa Rica. $R^2 = 0.41$

	d.f.	<i>F</i>	<i>P</i>
Treatment	2	74.14	< 0.0001
Location	3	35.40	< 0.0001
Treatment*Location	6	33.81	< 0.0001
Whole Model	11	42.21	< 0.0001

Table 6. Summary of non-ant visitors to *Passiflora vitifolia* during 16 5-minute observational periods of 21 shoots at successional plots in La Selva Biological Reserve, Costa Rica.

	Hymenoptera	Coleoptera	Diptera	Homoptera	Other	Total
Sugar	46	12	24	5	10	97
Nail polish	1	9	23	2	9	44
Control	5	15	18	3	10	51
Total	52	36	65	10	29	192

Butterfly diversity and abundance in primary and secondary tropical wet forest

Category: Independent Project
Participants: Susannah Nicholson and Michelle Hersh
Site: La Selva
Key words: Butterflies, butterfly diversity, Lepidoptera, Papilionidea, primary forest, secondary forest

Introduction

Long term monitoring of certain species can assist in identifying changes in biodiversity (Sparrow et al. 1994). Butterflies can serve as ecological indicators of habitat disturbance. Because the larval stage in most Lepidoptera is dependent on a specific host plant, species richness and abundance depend on host plant diversity (DeVries 1994). Therefore butterfly richness can indicate the amount of disturbance that has occurred in a habitat and measure its effect on diversity (Sparrow et al. 1994). Butterflies are also sensitive to changes in temperature, humidity, light, and vegetation structure; parameters that are frequently affected by habitat destruction (Sparrow et al. 1994).

Primary and secondary forests often differ in plant and insect diversity. Because a number of biotic and abiotic factors are involved, it is often difficult to predict apriori whether primary or secondary forest has a greater butterfly diversity. Primary forest offers undisturbed habitat to a number of Lepidoptera by promoting complex food web interactions (Gilbert 1980). For instance each species of Heliconiinae is associated with a different host plant species of Passiflora (DeVries 1994). When host plants are destroyed and the vegetation structure is altered as in secondary forests, certain Lepidoptera may no longer be able to exist. Butterfly

diversity might therefore be expected to be lower in secondary forest. In contrast, Lovejoy et al. (1986) document an initial decrease after disturbance but a subsequent increase surpassed original numbers of butterflies. They attributed this to an increase in light, which attracted more light tolerant butterflies and plants to the remaining forest while numbers in the undisturbed habitat remained constant (Lovejoy et al. 1986). In this case butterfly diversity was higher in secondary forest.

In this study, we investigated the differences in butterfly abundance and richness, host plant specificity, light preference, adult feeding preference and light levels between primary and secondary forest in order to gain insight on the disturbance and diversity in both habitats. We had no apriori predictions as to whether or not differences in butterfly diversity would exist between primary and secondary forests.

Methods

We conducted the study in primary and secondary growth of the tropical wet forest at the La Selva Biological Station in Sarapiquí, Costa Rica from November 19-December 4, 1998. La Selva is located in the Atlantic lowlands of Costa Rica. Mean annual rainfall is 3991 ± 748 mm and the mean annual temperature is 29.9 degrees. At least 479 species of butterflies have

been observed at La Selva (DeVries 1994).

We censused butterfly populations in two 50 m x 4 m transects of trail in both primary and secondary forests. The primary transect was located on the Sendero Sura near the Taconazo Creek about 100 m from the Arboleda. The secondary transect was located 250-300 m on the Sendero El Atajo near the Sura Creek. The section of secondary forest was clear cut in the 1970s for pasture land but has been growing since that time (McDade and Hartshorn 1994). La Selva purchased the land in 1981 as part of the Sarapiquí Annex (McDade and Hartshorn 1994). We used an Extech light meter (Extech Instruments, Inc., Taiwan) to obtain light measurements for every 2.5 m of transect in both forests.

We censused the butterflies in each transect by capturing individuals with aerial nets and by visual sightings. We started at opposite ends of each transect and walked from one end to the other to collect data and cover more area during the sampling time. We collected butterflies 2 m from the trail on both sides and only pursued individuals that entered the transect. We sampled for approximately one hour per day at each location alternating between transects to account for variation in light and climatic condition as well as variation in butterfly activity throughout the day. We collected data for a total of 12 hours, with 6 hours per location in 6 days.

We identified, marked, and released all butterflies that were. We noted the 5 m segment along the transect where each individual was caught or observed. Individuals that we could not catch were counted as visuals. Unless visuals were seen in groups, multiple sightings of a

particular species were only counted as one individual. We used DeVries (1987) to identify all individuals and to determine their host plant specificity, their light preference, and their adult feeding preference. We categorized species for host plant specificity as: a) generalist --if they fed on two or more host plant families b) specialists 1 --one family and two or more genera c) specialists 2 --one genera or d) specialists 3 --one species. In categorizing light preference, species were a) light loving, b) shade loving, or c) both. We classified adult feeding preference by subfamily as: a) nectar and/or pollen feeders or b) fruit, carrion, or feces feeders.

We constructed graphs to illustrate the number of species found in both transects and the numbers of species found in each 5 m range along the transects. We also constructed a graph of light levels along the transect and a species-time graph to determine if sampling time was adequate.

We calculated the Shannon-Weiner diversity index (H' : Begon et al. 1996) at subfamily and species level as well as subfamily and species evenness (J) for primary and secondary forests. We also calculated the percent similarity (S) of species between both forests (S : Todd Shelly pers. com.). The formula was: $S = C/(A + B) \times 100$, where C = the number of species in common between the two forests, A and B = the number of species found in primary and secondary forests respectively. We compared subfamily and species richness between primary and secondary forests using the Shannon-Weiner diversity index t -test comparison (Todd Shelly pers. com.). We used the JMP (SAS 1997) statistical package to perform a t -test comparing differences in light levels and χ^2 tests comparing

differences in host plant specificity, butterfly light preference, and adult feeding preferences between primary and secondary forests.

Results

We observed a total of 63 individuals: 43 in the primary transect and 20 in the secondary transect (Table 1). There were six different subfamilies with 17 species represented in the primary forest whereas the secondary forest contained six subfamilies and 11 species (Table 2). In both transects, we found most individuals in areas of high light. Of the four species that overlapped between forests, we found more individuals of those species in the primary forest (Figure 1). Individuals were not evenly distributed along either transect (Figure 2). The species-time curve started to plateau in the secondary forest but continued to increase in the primary forest indicating that are sampling time was not adequate (Figure 2).

The highest light meter readings in the primary forest occurred at 10 m and at 15 m in the secondary forest (Figure 3). We noted two light gaps in the primary transect, one from 0-15 m and a smaller one from 45-50 m. There were light gaps in the secondary transect from 15-30 m and from 40-50 m.

Subfamily and species evenness was slightly higher in the secondary forest (Table 2). The two transects were 28.6 % similar in species composition. The primary forest neither differed from the secondary forest in subfamily richness ($t = -0.268$, $df = 40.35$, $P > 0.05$) nor in species richness ($t = 0.597$, $df = 60.8$, $P > 0.05$). The primary and secondary forests did not differ in light levels, host plant specificity, light

preference, and adult feeding preference (Table 3).

Discussion

Our results and measurements of butterfly diversity in the two transects of primary and secondary forest at La Selva revealed that the two habitats were similar in butterfly diversity as well as in general habitat structure. The late successional stage of the secondary transect combined with the edge location of the primary transect may help explain why we found no differences in species richness, light levels, host plant specificity, light preferences and adult feeding preferences. However, because we observed differences in species abundance and composition between the primary and secondary transects, it is difficult to generalize about diversity in different habitats.

We found more individuals and species in the primary forest but little overlap in species composition between the two transects. Because butterflies are connected with different host plants in the larval stage and adults prefer certain plants for feeding, possible differences in the vegetation structure could cause differences in species composition. Competition among Lepidoptera, variation in levels of predation and defense strategies such as crypsis and aposematic coloration may also account for differences in species composition and abundance. Our ability to see certain aposematically colored butterflies such as the Ithomiinae may have caused us to capture more of these individuals than butterflies with cryptic coloration. Three of the four species that overlapped between forests were in the subfamily Satyrinae. Most Satyrids are generalists and prefer undergrowth

associated with light gaps (DeVries 1987). We found light gaps in both transects which may provide habitat for Satyrids in both forests. As generalists, Satyrids do not require a specific vegetation which suggests that they could exist in both habitats.

The light levels did not differ in the primary and secondary transects. In both transects we observed a high variation in light due to light gaps formed by fallen trees and deep shade from dense canopy. High species numbers corresponded with high light levels. Many of the species that we found were light tolerant species that are attracted to light gaps. These species may find their larval host plants and adult feeding plants in these gaps.

The species time curve started to level off in the secondary forest indicating that our sampling was adequate to give a good representation of the species found in that area. However, the curve for the primary forest has not plateaued so it is possible that the data are incomplete. Had we continued sampling, we may have obtained a more accurate representation of the species abundance and diversity. Butterflies exhibit vertical stratification within the forest (DeVries 1988), which we were unable to census and include in our study. A more thorough study would include baited traps, in addition to aerial nets, to attract and census species from higher stratifications in order to examine the diversity at all forest levels rather than just in the understory.

There was a more even distribution of species in the secondary forest than the primary. Several species seemed to dominate in the primary forest. For example, we observed a large number of *Heliconius sapho leuce*, which exhibit a pupal mating system, in the primary forest.

In a pupal mating system, males flock to the host plants to mate with females emerging from the pupae (Erika Deinert pers. com.). Because *H. sapho leuce* have a pupal mating system and are specialists 3, we may have observed more *H. sapho leuce* individuals if their particular host plant was located in our transect. However, we recalculated the Shannon-Weiner index and the evenness ($H' = 1.047$, $J = 0.673$) excluding *H. sapho leuce* and we found no differences, therefore suggesting that the large number of *H. sapho leuce* did not affect our diversity or evenness results.

Even though there was a low percentage of similarity between primary and secondary forests, the two transects did not differ in subfamily or in species richness. The forests are considered primary and secondary but upon closer examination, the forests may have been more similar than we realized. The secondary transect was located in a late successional forest and the primary forest may have edge effects because it was located near one of the entrances to the Arborleda, a disturbed habitat. Edge effects can influence species composition in similar ways as disturbance. Edge habitat allows invader species, often generalists, to enter the forest and exist and compete with interior species (Noss and Csuti 1997).

We also found no differences in host plant specificity, light preference, and adult feeding preference between the two transects. Because there was no difference in light levels between the two forests, it follows that light preference would not differ since the butterflies would have access to similar light environments within transects. Similarities between habitats could allow comparable host plant species to grow in both areas and attract similar numbers of

generalists and specialists. Similar food sources would allow equal numbers of nectar/pollen feeders and fruit/carrion/feces feeders in both transects.

In conclusion, we observed no differences in butterfly diversity between primary and secondary forests whereas the two forests differed in species abundance and composition. Butterfly diversity may not differ between these two habitats. However, it is possible that this reflects a similarity between habitats and indicates that edge primary forest may be similar in diversity to a late successional secondary forest. Our results demonstrate that it is difficult to generalize about diversity among habitats including primary and secondary forest. Instead, different successional stages may be required to maintain butterfly diversity.

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Table 2: Butterfly species diversity and evenness based on the Shannon-Weiner diversity index for two 50 m tropical wet forest transects in La Selva Biological Station, Sarapiquí, Costa Rica.

Forest Type	Diversity (H') by subfamily	Evenness (J) by subfamily	Diversity (H') by species	Evenness (J) by species
Primary	0.583	0.357	1.036	0.643
Secondary	0.608	0.467	0.989	0.760

Table 3. Mean light levels in two 50 m tropical wet forest transects in La Selva Biological Station, Sarapiquí, Costa Rica.

Forest Type	Mean light level (lux)	St. Dev
Primary	1031.19	838.07
Secondary	1326.67	574.28

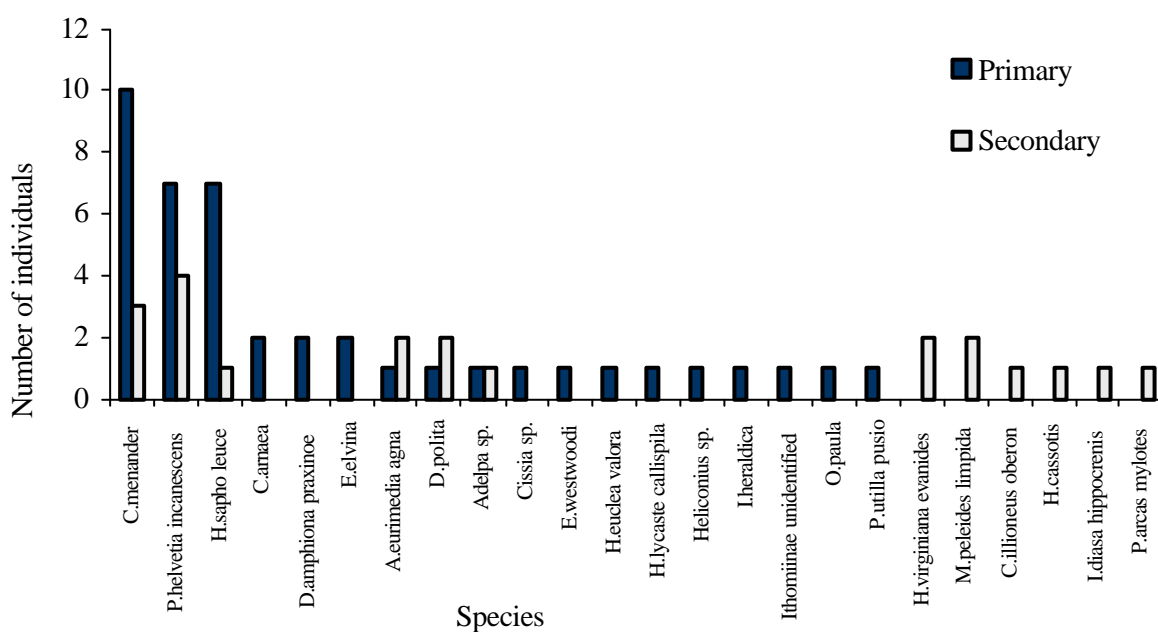


Figure 1: Distribution of individuals of the different butterfly species found in two 50 m tropical wet forest transects at La Selva Biological Station, Sarapiquí, Costa Rica.

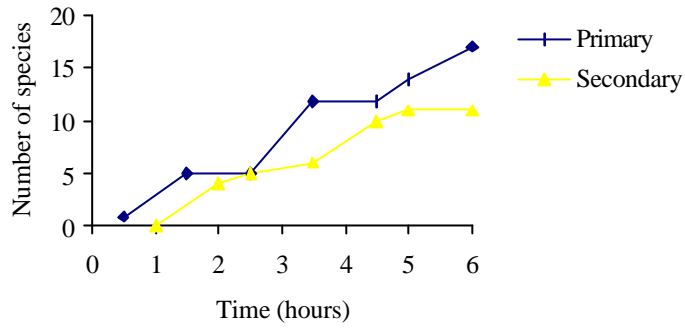


Figure 2: Species-time curve for the number of different butterfly species collected in two 50 m transects of tropical wet forest at La Selva Biological Station, Sarapiquí, Costa Rica.

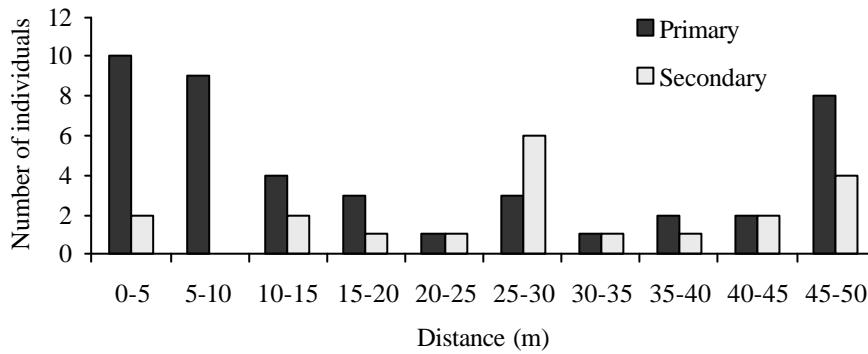


Figure 3: Location of butterflies captured or observed along two 50 m rainforest transects at La Selva Biological Station, Sarapiquí, Costa Rica.

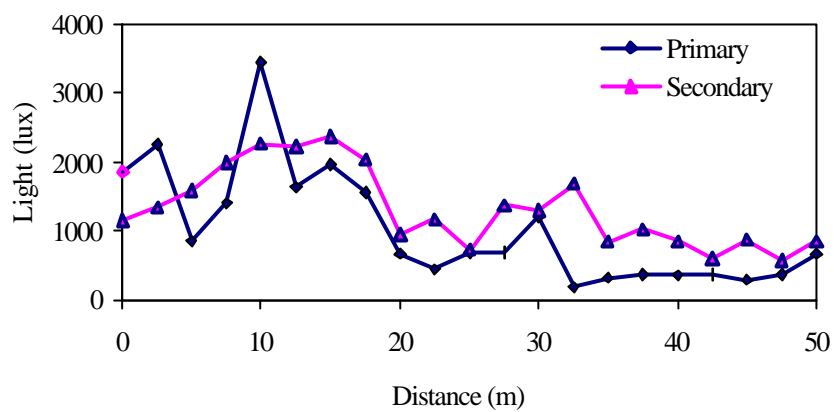


Figure 4: Light levels along two 50 m rainforest transects at La Selva Biological Station, Sarapiquí, Costa Rica. Readings were taken on a slightly overcast day.

Temporal and spatial memory in the giant tropical ant, *Paraponera clavata*

Category: Independent Project
Participants: Lisa Stano and Margaret Yu
Site: La Selva
Key words: Learning, *Paraponera clavata*, spatial orientation, temporal memory

Introduction

The ponerine ant *Paraponera clavata* employs several strategies to exploit localized food sources effectively. Foragers often rely heavily on pheromonal cues (Breed & Bennett 1985) and demonstrate a system of graded recruitment in response to food quality, food quantity and distance of resource to colony nest (Breed et al. 1987). Local ground cues can also be important for short-range orientation to food sources (Harrison et al. 1988), and visual landmarks are particularly essential to long-range ground foragers (Baader 1996). In addition to these sensory-based foraging mechanisms, Harrison and Breed (1987, as cited in London 1992) demonstrated that *P. clavata* has a capacity for temporal learning. Ants were trained to arrive at a feeding site of sugar solution at specific times of day, exhibiting temporal memory for the simulated nectar source. In this study, we further explore both temporal and spatial memory capabilities in *P. clavata*. We hypothesized that 1) as a colony, *P. clavata* will show stronger temporal memory for higher-quality nectar rewards; and 2) individual foragers led repeatedly to a simulated nectar source will be able to relocate the site in the absence of a pheromone trail, thus indicating short-term spatial memory for nectar source location.

Methods

We performed this study at the La Selva Biological Station, Heredia Province, Costa Rica, from 20 November to 6 December 1998. Nests of *Paraponera clavata* normally occur at the bases of large trees, and workers forage primarily in the canopy of the host tree or adjacent trees for nectar or arthropod prey (Young & Hermann 1980). The cleared understory of the La Selva arboretum allowed easy access to nests of *P. clavata*.

Temporal memory

To examine temporal learning, we located a *P. clavata* colony nest at the base of *Warszewiczia coccinea* (Rubiaceae) and performed two trials lasting 6 and 7 days respectively. Though foraging activity of *P. clavata* generally peaks during the night, the colony was active throughout the day, enabling daytime experimentation. We performed the first trial from 20-25 November between 1600 and 1800 h each day. Two feeding sites were established on the trunk 14 cm on either side of the main foraging trail and 98 cm from the ground. To verify that sites were far enough from the main foraging trail to avoid random ant visitation, we observed ant activity for 30 minutes; no ants approached either feeding site during this time. Each feeding site consisted of a vial cap (diameter 2.2 cm, depth 0.8 cm) tacked securely to the bark throughout the entire trial. For the same 30-minute period on four consecutive days, we filled the sites

with 30 drops of sugar solution, and washed out the vial caps at the end of each period. The feeding site on the left side of the foraging trail was designated as “low-quality” and filled with 0.2 M sugar solution. The other site was designated “high-quality” and filled with 1.0 M sugar solution. We led a different ant to each site at the start of the 30-minute period, using a drop of sugar solution at the tip of an eyedropper. If ants were not recruited to the simulated nectar source within 5 minutes, we led a second ant. We recorded the number of ant visits during this 30-minute training period. An “ant visit” occurred when an ant came to a feeding site and collected a visible drop of solution between its mandibles.

The four training days were followed by two test days during which no sugar sources were provided. During the test days, we redefined an “ant visit” as when an ant crawled onto the feeding site or began probing the inside of the vial cap with its antennae. Each test day, we recorded the time of each ant visit over a 90 minute interval: 30 minutes before the supposed feeding time (“pre-feed” period); the 30 minutes during which ants had been provided with sugar on previous days (“feed” period); and 30 minutes following (“post-feed” period).

The second trial occurred from 30 November to 6 December and was identical to the first except for the following modifications. First, because an *Azteca* ant colony had interfered with *P. clavata* attempts to feed at both sugar sites and particularly at the “high-quality” feeder, we relocated the sites to 21.5 cm on either side of the main foraging trail and 118 cm from the ground. To create an even more distinct difference in resource quality, we

increased the sugar concentration of the “high-quality” feeder to 2.0 M. Also, the 30-minute observation periods of trial 1 test days appeared insufficient for adequate comparison of ant visits (see results). Therefore, in addition to recording data for the three 30-minute periods, we decided to compare each entire 90-minute segment to a 90-minute control segment earlier in the same day (1030 to 1200 h). For each 90-minute segment, we recorded both the time of ant visits to feeders and the “ant traffic.” We measured ant traffic by recording the number of times ants crossed the horizontal line between the two feeders. This allowed us to compare the percentage of foraging ants that visited the feeders rather than the absolute number of ant visits, which may be affected by variation in ant activity. We also recorded data for a third test day. However, ant visitation on this day was relatively equivalent between the two 90-minute intervals (see results), likely indicating loss of memory. We therefore did not include these data in statistical analyses.

A paired *t*-test compared ant visitation to the high and low-quality sites during feeding days in trial 1, trial 2, and when data from the two trials were combined into one set. A two-way ANOVA was performed on the entire set of test days (i.e., trials 1 and 2 combined) to determine if resource quality affected ant visitation, or if ant visitation differed between successive test days. This analysis was performed on each type of 30-minute interval (i.e., “pre-feed,” “feed” and “post-feed” periods). Since neither resource quality nor test day affected ant visitation (see results), we combined data points for each feeder and test day into one data set. The Kruskal-Wallis test was then used to

determine if number of ant visits on test days differed significantly between pre-feed, feed and post-feed periods. For trial 2, a *t*-test compared the percentage of foraging ants that visited the feeders during the entire 90-minute test periods and the control periods. All statistics were performed using the *JMP* analysis program (Sall & Lehman 1996).

Spatial memory

We located a different colony of *P. clavata* to explore short-term spatial memory for nectar sources. To train individuals to a specific food site, we placed two drops of 2.0 M sugar solution on the bark of the tree, at least 0.5 m away from the main foraging trail to minimize interference from other foraging ants. We led a foraging ant to the sugar source using solution at the tip of an eyedropper and allowed it to feed for 3-5 seconds. We then placed a funnel over the ant and slid the funnel horizontally 13-16 cm away from the sugar source to a "starting point." We removed the funnel when the ant was oriented in the direction of the food source, and led the ant directly to the source. We allowed the ant to feed for 3-5 seconds and repeated the above procedure, leading the ant for a total of three times from starting point to food source. If an ant could not be led for three consecutive times, we discontinued further experimentation with the ant.

If the ant was led successfully, we proceeded with testing of spatial memory. When the ant completed the third lead, it was again allowed to feed for 3-5 seconds. We then moved the ant with the funnel to the same starting location. When the ant was oriented in the direction of the food source, we removed the funnel but did not lead it upon release. If the ant relocated the

source, it was allowed to feed as above. This was performed three times. We then moved the ant with the funnel as before, but released it (unled) when oriented in the opposite direction. If the ant relocated the source, it was allowed to feed as above. This was also performed three times. Because ants tended to move in the direction of release, variation in release direction corrected for random discovery of the food source. Also, because a previous study found that food rewards were unlikely to be discovered at distances of more than 10 cm (Breed et al. 1996), the 13-15 cm displacement was assumed to be great enough to prohibit random discovery of the reward. In none of the trials was the ant observed to drag its gaster along the substrate, which would indicate pheromonal marking (Breed & Bennett 1985). Thus successful completion of the test would indicate use of spatial orientation and memory.

If the ant successfully completed the six testing trials, we recorded a "positive" response. If the ant failed to relocate the source within 1 minute of release in any of the six trials, we recorded a "negative" response and at that point the experiment was discontinued with the ant.

Results

Temporal memory

The number of ant visits did not differ significantly between the high and low-quality sites during the feeding days of either trial 1 or trial 2, or when the feeding days from each trial were combined (Table 1). Ant visitation also did not differ between the two test days or between the high and low-quality feeders, when analyzed within each 30-minute period (Table 2). Therefore, we could include

both feeders and all the test days when comparing ant visitation between pre-feed, feed and post-feed intervals. No significant difference was found in ant visitation between any of the intervals (Kruskal-Wallis: $df = 2$; $\chi^2 = 3.7035$; $P = 0.1570$).

However, because we did not expect ants to arrive perfectly on time for the feed interval, we subdivided the 30-minute intervals into 10-minute periods for a more detailed breakdown of ant visitation. This higher resolution allowed for anticipation of the feed interval and inexact timing. A bar graph of the 10-minute periods (trials 1 and 2 combined) reveals relatively high ant visitation just prior to the test period. This graphic depiction more accurately characterizes the 90-minute interval: low numbers of ants visited the feeders for the majority of the pre-feed period, and ant visitation increased sharply as the feed period approached (Figure 1). The 90-minute test intervals of trial 2 included this anticipation. Consequently, mean ant visitation during the 90-minute test intervals was significantly greater than during the control intervals (test/control ratio of ant visitation = 3.46, $SD = 2.05$, Table 3). As noted in methods, data from the third test day of trial 2 were not included in any of the statistical analyses, since visitation during the test and control intervals was relatively equivalent (test/control ratio = 1.31, $SD = 0.14$).

Spatial memory

Out of approximately 50 *P. clavata* individuals, 21 were led successively for three consecutive times to the sugar solution site. Of these, 13 individuals (61.9 %) gave a positive response by completing all trials, and 8 individuals (38.1 %) gave a negative response.

Discussion

Our results provide further evidence for temporal memory in *Paraponera clavata*. Though ants did not arrive precisely at the start of the 30-minute feeding interval on test days, breakdown into 10-minute periods reveals that ants anticipated the feeding time. Also, while ant visitation on test days did not decrease immediately after the feeding time, this is likely due to incomplete emptying of vials. Remnant sugar left in vials at the end of the training period would cause ants to continue visiting the sites during both training and test days. Comparison of broader, 90-minute intervals corrected for inexact timing of ants and incomplete sugar solution removal. A greater proportion of foraging ants visited the sugar sources during the 90-minutes containing the feeding time than during the control interval. This indicates that *P. clavata* individuals can remember when nectar resources will be available at a particular site, an ability that would allow more efficient exploitation of this cyclically available resource (London 1992). Evidence of temporal learning is consistent with studies performed by Harrison and Breed (1987, as cited in London 1992), in which ants were trained to return to a sugar water site at specific times of day.

We further proposed that the colony would show greater memory for a high-quality sugar source than a low-quality source. Because *P. clavata* exhibits graded recruitment in response to resource quality (Breed et al. 1987), we assumed that more ants would visit the high-quality solution during training, resulting in greater visitation during test days. The results contradicted our predictions, indicating equivalent visitation regardless of resource

quality. However, recruitment to each feeder was also unexpectedly equivalent. This is likely due to large numbers of *Azteca* sp., a small but highly aggressive species of ant, which often swarmed the high-quality feeder and prevented *P. clavata* individuals from reaching this resource during training days of trial 1. If *Azteca* had not interfered with recruitment to the high-quality feeder, more ants would have visited the feeder on training days and likely on test days as well.

Nonetheless, our results may also suggest that individual ants remember all food sources equally, regardless of quality. This would cause equivalent visitation to sugar sites on test days, assuming equivalent recruitment on feeding days. However, because *Azteca* interference greatly affected our results, variation in memory strength among individuals should not be disregarded. Differences in resource quality may cause differences in memory strength: high-quality sites may elicit stronger and more lasting memory than low-quality sites. This could be investigated by examining the decrease in the number of ant visits through successive test days. If memory for a high-quality site is longer-lasting, ant visitation will decrease at a lower rate for a high-quality site than for a low-quality site. Future studies could examine this by marking individual ants and observing the numbers that returned to the site across test days.

As we expected, some *P. clavata* individuals also showed the capability to remember location of sugar sites without the aid of pheromonal cues. This indicates accurate short-term spatial orientation and memory, at least for nearby sugar sources. However, the extent to which spatial memory depends on visual cues or an

innate sense of direction is unclear. Though the surface of the trunk was highly uniform in both color and texture, and visual cues were absent from the sugar site, the ants may have been able to discriminate between similar bark surfaces to relocate the source. Ants might also relocate sites simply by remembering the correct direction to locate the reward; this may be more likely, as ants that gave a “positive” response moved rapidly and immediately to the sugar site, apparently without methodical evaluation of surroundings.

However, though *P. clavata* appears to have the capacity for spatial orientation and memory, the majority of ants could not relocate the sugar site. Because all tested individuals were actively looking for food prior to manipulation, we presumed that the sugar source would be equally valuable to each individual. Thus differences in desire to relocate the reward were not likely. Other sources of variation may explain the ability of only some ants to relocate the sugar site. Individuals may vary both in capacity to remember site location and in foraging experience. Better spatial memory and/or greater foraging experience (Baader 1996) may enable some individuals to better remember visual cues or direction.

Furthermore, type of foraging activity may influence spatial memory capabilities and utilization of visual cues. Baader (1996) demonstrated that ground foragers, particularly individuals that foraged for long ranges, relied heavily on visual landmarks for orientation on the forest floor and relocation of the colony nest. Harrison et al. (1988) also found that ants foraging near the tree base preferred to use visual ground cues than pheromone trails. Canopy foragers, on the other hand,

likely depend on both pheromonal and visual cues for short-range orientation to food sources (Harrison et al. 1988). Pheromone trails may be particularly essential for travel on trunks and branches, where distinct visual landmarks are lacking. Because all individuals we tested were canopy foragers found on the trunk, spatial orientation and ability to remember visual cues may be relatively unimportant for these individuals. Experimentation with a greater sample size of both canopy and ground foragers would clarify the importance of spatial memory in different types of foraging activity.

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Table 1. Results of paired *t*-test comparing number of *Paraponera clavata* visits to high and low-quality sugar solutions during training, at La Selva Biological Station, Costa Rica.

	<i>n</i>	Mean number of visits	SD	<i>t</i> -test	<i>P</i> -value
Trial 1 (High)	4	36.79	10.08	1.5988	0.2082
(Low)	4	61.00	25.07		
Trial 2 (High)	4	75.75	16.42	3.1485	0.0513
(Low)	4	28.75	13.72		
Overall	8	56.25	24.36	0.6818	0.5173

Table 2. Results of two-way ANOVA examining effects of sugar solution quality and test day on the number of *Paraponera clavata* visits to feeding sites, at La Selva Biological Station, Costa Rica. Ants were observed at the same time on two test days following feeding days. Results are given for three intervals: 30 minutes before the supposed feeding time (“pre-feed”); the 30 minutes during which ants had been provided with nectar during feeding days (“feed”); and 30 minutes following (“post-feed”).

	<i>n</i>	<i>P</i> -value	F-ratio
Pre-feed (Quality)	8	0.7878	0.0806
(Day)	8	0.7878	0.0806
Feed (Quality)	8	0.4810	0.5792
(Day)	8	0.2488	1.7021
Post-feed (Quality)	8	0.5909	0.3293
(Day)	8	0.1667	2.6157

Table 3. Results of *t*-test comparing percentages of foraging *Paraponera clavata* ants that visited feeders during two 90-minute intervals: the test period containing the feeding time to which the ants were trained and the control period earlier in the day. Study was performed at La Selva Biological Station, Costa Rica.

	<i>n</i>	Mean % Visitation	Standard error	<i>t</i> -test	<i>P</i> -value
Test period	4	4.5556	0.544	3.852	< 0.0084
Control period	4	1.5921	0.544		

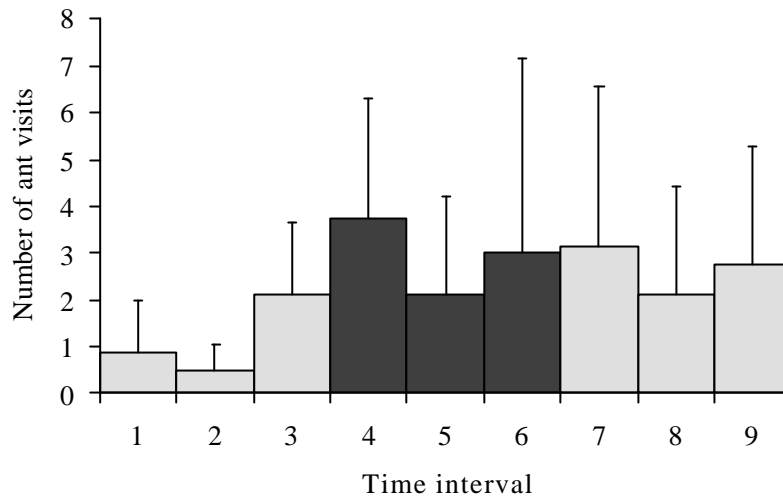


Figure 1. *Paraponera clavata* visitation to feeding sites over a 90-minute period, on days when sugar solution was not provided. The 90-minute period is subdivided into nine 10-minute intervals. Each bar represents the mean number of ants that visited the feeding sites during the 10-minute interval. Intervals 4-6 represent the 30-minute period during which the ants were provided with sugar solution during feeding days. The 30-minute periods before and after this feeding time are represented by intervals 1-3 and 7-9, respectively. Study was performed at La Selva Biological Station, Costa Rica.

Food choice and foraging behavior of ants

Category: Independent Project
Participant: Cory Tyszka
Site: La Selva
Key words: Ants, foraging, Formicidae, pheromones, recruitment

Introduction

Ants (family Formicidae) are a major component of biota in the tropics. They comprise nearly one third of the animal biomass of the forests (Erika Deinert, pers. comm.). They are social insects, and use chemical signals within their colonies for purposes of emigration to new sites, new nest construction, defense of the colony, and to communicate information about food sources (Hölldobler and Wilson 1990). Communicating about food sources for retrieval purposes is called recruitment. Ants use pheromones for recruitment, as well as visual displays. Pheromones are chemicals secreted by exocrine glands near the tip of the abdomen in ants (Bradshaw and Howse 1984). There are two forms of recruitment. One is a more primitive form, called tandem-running. This is the process by which the scout of the ant colony returns to the nest after finding a food source and recruits a single worker. As they go back and forth between the nest and food source, pheromones are laid on the ground, and more ants are recruited (Preston-Mafham and Preston-Mafham 1993). A more advanced form of recruitment is a mass recruitment system. This system is one in which a scout finds a food source, then reports back to the nest leaving a pheromone trail from the food source to the nest. At the nest, some species perform “waggle” signals, which cues other individuals to follow, and a number of ants

leave the nest in search of the food source. The initial number of workers is typically too high for the amount of food due to crowding, so some ants leave without leaving a trail. The ants that successfully get the food return to the nest leaving a trail since trails tend to last only a few minutes (Hölldobler and Wilson 1990). Whereas the primitive system recruits one ant at a time, the more advanced system of recruitment attracts many ants at once. In addition to attracting nest mates, pheromones also function to orient others to the food source (Bradshaw and Howse 1984). Hölldobler and Wilson (1990) also state that ants have the ability to control the number of individuals recruited by the amount of trail pheromones excreted.

This study focuses on recruitment behaviors of ants based on food quality and quantity. The two hypotheses of this study are that 1) ants will prefer protein to carbohydrates, and that 2) recruitment will be higher to high density food sources than to low density food sources. I predict ants will choose protein over carbohydrates because in general, protein is found in insects, which requires that the ants locate and then kill their prey. This takes more energy than returning to a known nectar source. Similarly, since ants have been shown to control recruitment rates, I predict they will signal for higher recruitment at high-density food sources.

Methods

The study site was the arboleda at La Selva Biological Reserve, Sarapiquí, Costa Rica. Sugar and tuna fish were used as food baits, each at 1g and 2g densities. The sugar served as a source of carbohydrates while the tuna served as a protein source. For each trial, bait was weighed in dishes and placed approximately one foot off the trail at random, unique locations in the arboleda. The sets of four dishes were placed equidistant from the trail in a row with about one cm between dishes. The order of dishes alternated between tuna fish and sugar to clearly separate the different densities. Each of the trials had a duration of one hr. All trials were performed between 23 November and 2 December 1998 between the hours of 7:30 am and noon due to decreased afternoon activity. I recorded the time of the first arrival to each dish to see if response time varied between treatments to see which baits were most attractive to the ants. Thereafter, the number of individuals of each morphospecies to visit each dish was recorded, along with the time they visited, to measure recruitment rates. After one hour, the dishes were taken back to the lab and weighed to calculate the weight taken or eaten from each dish.

Number of visits to tuna or sugar, and number of visits to high or low densities were compared for four morphospecies of ants with chi-square tests performed by hand. Time of first arrival to each dish was compared between baits and density treatments with nonparametric Wilcoxin rank sum tests, and *t*-tests were used to compare weight of bait taken between high and low density treatments for tuna and sugar using JMP (Sall and Lehman 1996).

Results

There were four most common morphospecies that visited the dishes, species of the genera *Ectatomma*, *Aphaenogaster*, and two species of *Pheidole*, denoted *Pheidole* red and *Pheidole* black. *Ectatomma* are large black ants, *Pheidole* red are tiny red ants, *Pheidole* black are small black ants, and *Aphaenogaster* are large red ants. Two morphospecies, *Pheidole* red and *Aphaenogaster*, showed no preference for tuna or sugar, however *Ectatomma* preferred tuna while *Pheidole* black preferred sugar ($\chi^2 = 22.5$, $df = 3$, $P < 0.05$; Table 1). *Ectatomma*, *Pheidole* red, and *Pheidole* black all preferred high densities of bait, while *Aphaenogaster* preferred low-density baits ($\chi^2 = 109.4$, $df = 3$, $P < 0.05$; Table 2). Overall there was no difference in the number of individuals visiting tuna vs. sugar (Table 1). Interestingly, however, 2.8 times more individuals visited high-density baits than low density baits even though there was only 2 times more bait in the high-density dishes (Table 2). Weights of tuna and sugar taken or eaten did not differ among density treatments (Table 3). Initial time to arrival was not related to bait or density treatment (Table 4). Mean arrival times were, however, quicker for high-density dishes than low-density dishes, and also quicker for tuna than sugar (Table 5). The cumulative number of individual visits to the dishes was highest for *Pheidole* red and lowest for *Aphaenogaster* (Figure 1).

Aphaenogaster took larger chunks of tuna than did *Ectatomma*. *Ectatomma* and *Aphaenogaster* also varied in the manner in which they consumed the sugar. *Ectatomma* stayed in the dish and ate the sugar, while *Aphaenogaster* rolled the

sugar into balls and took the balls away. In addition, *Ectatomma* initially visited the tuna dishes, but switched to sugar later during the trials, usually in the second half of the hour. Another interesting observation was that both *Ectatomma* and *Aphaenogaster* occasionally jumped away from dishes when those dishes contained either *Pheidole* red or *Pheidole* black. These smaller ants perform phragmosis, which is the process of keeping others out of an area by pushing them out with their heads (Hölldobler and Wilson 1990). The two large morphospecies, *Ectatomma* and *Aphaenogaster*, sometimes shared the task of carrying back large chunks of bait with another individual of the same species. This occurred when one individual was clearly having difficulty picking up and carrying a chunk. In addition to the four morphospecies in this study, there were at least four other species of ants that visited the dishes, but at a much lower density. Other visitors to the tuna baits included Coleoptera, Diptera, and Orthoptera.

Discussion

The data show equivocal support for the hypotheses that ants will prefer tuna to sugar, and that ants will have higher recruitment to higher densities. Bait and density preference was dependent upon the species visiting the bait. Bait preference could depend upon the energy needs of the particular species. *Ectatomma*, who chose tuna, may need a higher protein diet due to colony activities, while *Pheidole* black may need less protein because of its small size. *Aphaenogaster* preferred low-density treatment whereas the other three species visited high-density treatments more often. There may be sufficient bait for *Aphaenogaster* in the 1g samples so it can

specialize on the lower-density baits. For small species such as *Pheidole* red and *Pheidole* black, larger samples may allow for higher recruitment due to the larger area covered by the bait, thereby resulting in more room for collecting food since recruitment is partially due to availability of space for individuals (Bradshaw and Howse 1984). Even though there was no relationship between mg of bait taken and density of bait in the dish, cumulatively more mg of high-density bait was eaten than low-density bait. For tuna, 71.0 % more weight was taken or eaten from the 2g sample than from the 1g sample. Likewise, 62.2 % more sugar was eaten from the 2g sample than from the 1g sample. Arrival times were not related to bait choice with respect to bait type and bait density. This could be due to the close proximity of all the dishes to each other.

There were differences in recruitment for different species over time. *Ectatomma* exhibited a slow and steady initial recruitment, but recruitment rate increased at 20 minutes. *Pheidole* red had high recruitment overall, but at 15, 25, and 35 min, recruitment levels off for those 5-min intervals. At 40 min, *Pheidole* red seem to have equilibrated the number of individuals visiting the baits, sending no extra individuals who cause plateaus in the slope of the graph by not returning. *Pheidole* black show a slow initial recruitment with an increase in number of individuals after 35 min for 10. *Aphaenogaster* showed a slow increase in number of visitors to the baits. They had relatively constant recruitment for the full 60 min. Recruitment also varied with bait (Figures 2 and 3). *Pheidole* black showed a jump in number of visitors, but then the number leveled off for tuna, however

recruitment continued to sugar after the initial rise in recruitment. *Ectatomma* showed steady recruitment to tuna from early on, but barely visited sugar until 25 min, and then had a slightly lower slope than to tuna. Neither *Aphaenogaster* nor *Pheidole* red show much difference in recruitment to tuna or sugar.

The difference in slopes of the ants may reflect variation recruitment strategies. *Pheidole* black may have a primitive recruitment method, starting out tandem-running and therefore leading to a slow, steady increase in visitation. Then, when enough of a pheromone trail is in place, a significant increase in visitation results, presumably when the pheromone trail had been fully created. *Pheidole* red, on the other hand, may use a mass recruitment system, evident by a few initial visitors to the food sources, then a steep increase in the number of visitors. Mass recruitment may be beneficial to this species because they tend to forage in large groups. These groups tend to stay and eat at the food source instead of bringing the food back to the nest as *Ectatomma* and *Aphaenogaster* do. Because of this, it is most efficient to the ants to have a mechanism to communicate food sources to the entire nest at once instead of wasting time, and therefore energy, by going back and forth between the nest and the food source slowly recruiting individuals. Recruitment of *Ectatomma* may result from tandem-running, with slow initial recruitment, and then an increase in recruitment rate. Likewise, *Aphaenogaster* recruitment may be tandem-running recruitment, with a steady increase in visitors for the entire duration of the trials. This steady increase without a

change in slope may also be partially due to a limited population size, where all available individuals are repeatedly visiting the food sources.

At La Selva Biological Reserve, ant recruitment patterns seem to vary among species. Each species seems to have a unique recruitment system within the two major types which corresponds to foraging habits and population sizes of the species. In general, ants eat tuna and sugar equally, however they tend to be attracted to larger amounts of baits.

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Table 1. Numbers of visitors to each bait for each species of ant at La Selva Biological Reserve, Costa Rica.

	<i>Ectatomma</i>	<i>Pheidole</i> red	<i>Pheidole</i> black	<i>Aphaenogaster</i>	Totals
Tuna Fish	181	271	80	32	564
Sugar	112	269	115	39	535
Totals	293	540	195	71	1099

Table 2. Numbers of visitors to high and low bait density treatments for each species of ant at La Selva Biological Reserve in Costa Rica.

	<i>Ectatomma</i>	<i>Pheidole</i> red	<i>Pheidole</i> black	<i>Aphaenogaster</i>	Totals
High	179	464	138	28	809
Low	114	76	57	43	290
Totals	293	540	195	71	1099

Table 3. Summary of *t*-tests comparing high and low density baits with respect to mg of bait taken by ants at La Selva Biological Reserve in Costa Rica.

	<i>t</i> -test	df	SE	<i>P</i>	Density	Mean	SD
Sugar	0.692	10	41.2	0.5048	Low	45.8	53.8
					High	74.3	85.4
Tuna	0.543	10	86.5	0.5987	Low	159.5	150.5
					High	206.5	149.1

Table 4. Results of Wilcoxin rank sum test comparing the effects of type of bait and density of bait with time to first arrival by ants at La Selva Biological Reserve in Costa Rica.

		<i>Z</i>	<i>P</i>
Sugar vs. Tuna	Low	-0.721	0.4712
	High	-1.041	0.2980
Low vs. High	Sugar	-0.400	80.689
	Tuna	-0.881	0.3785

Table 5. Means and standard errors of time, in minutes, of first arrival of ants at four types of baits: 1) low-density tuna, 2) high-density tuna, 3) low-density sugar, and 4) high-density sugar at La Selva Biological Reserve in Costa Rica.

	Tuna		Sugar	
	Mean	SE	Mean	SE
Low	11.82	3.13	16.11	4.34
High	10.22	4.69	13.34	2.18

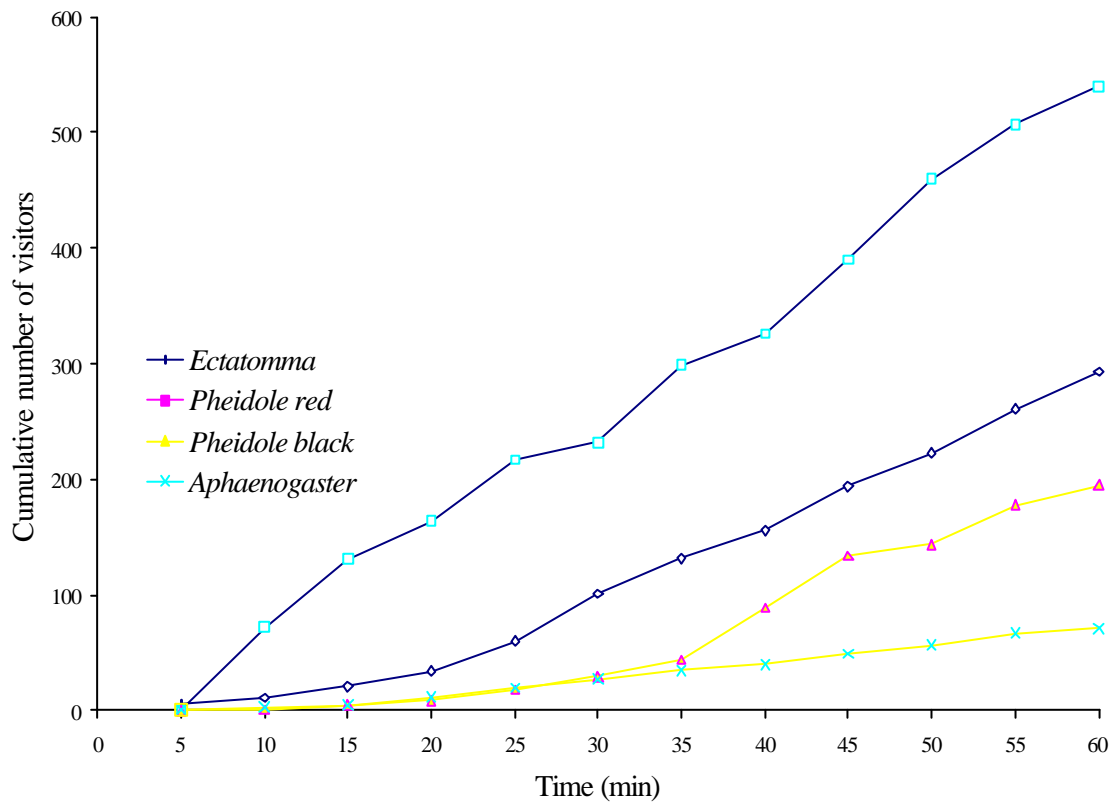


Figure 1. Cumulative number of visitors of four ant morphospecies to tuna and sugar baits at La Selva Biological Reserve in Costa Rica.

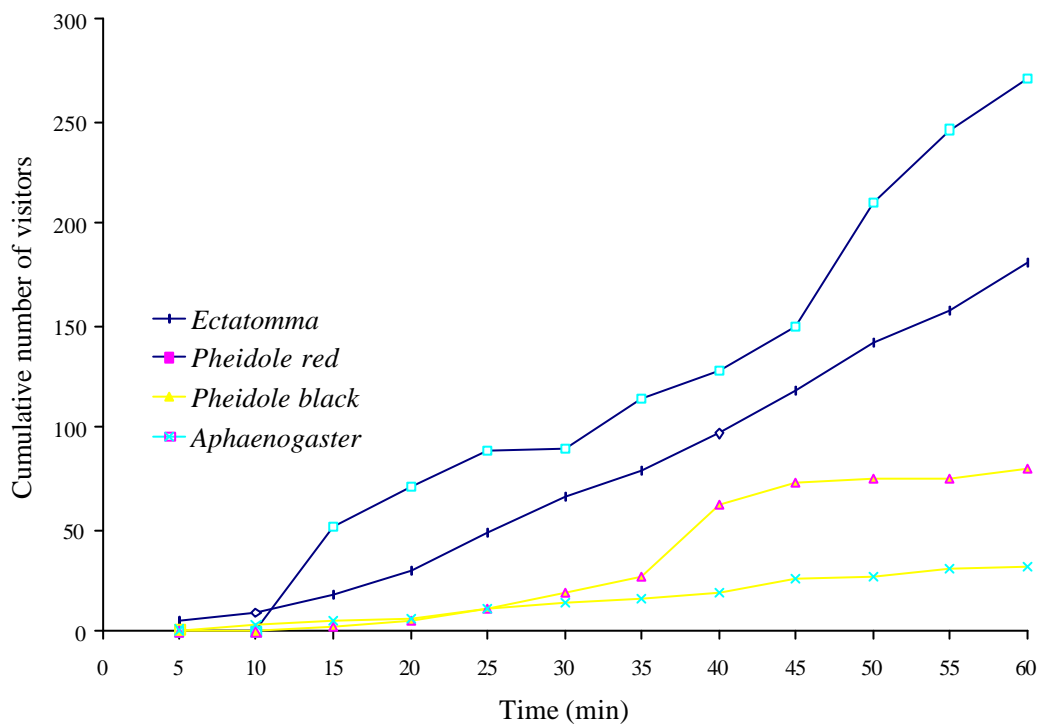


Figure 2. Cumulative number of visitors of four ant morphospecies to tuna baits at La Selva Biological Reserve in Costa Rica.

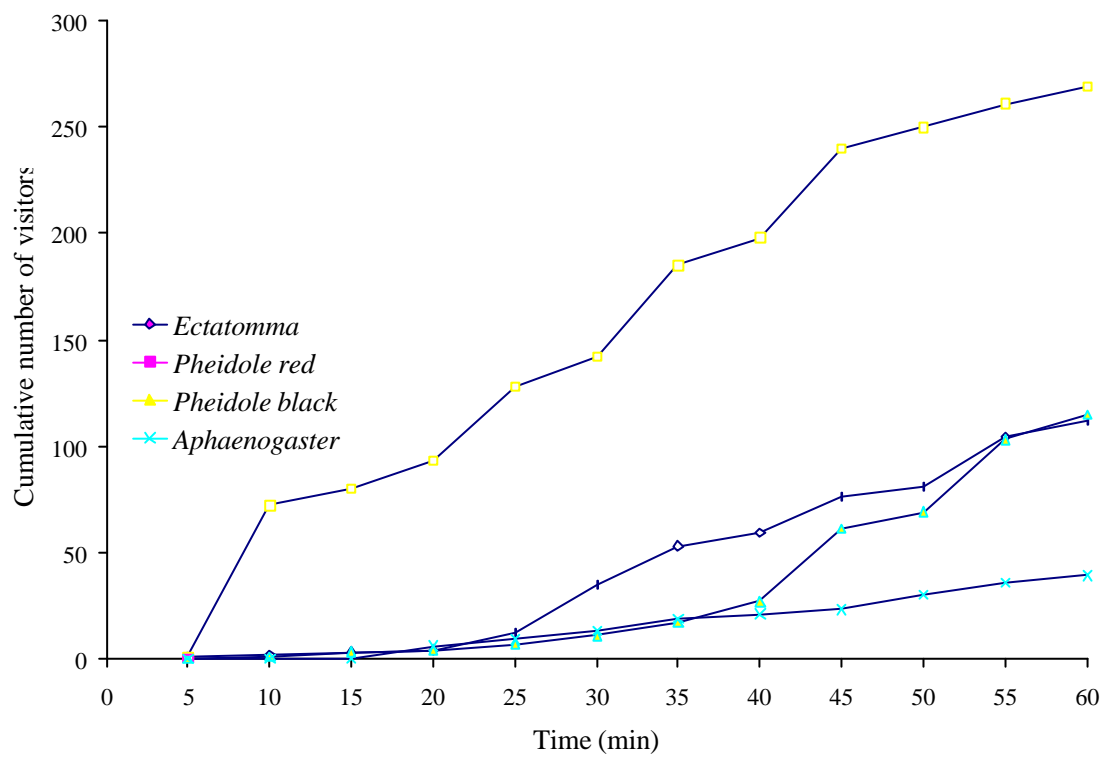


Figure 3. Cumulative number of visitors of four ant morphospecies to sugar baits at La Selva Biological Reserve in Costa Rica.

Rhizobium nodulation of *Pentaclethra macroloba* seedlings in varying light regimes

Category: Independent Project
Participants: Raivo-Erik René Vihman and Athena Dodd
Site: La Selva
Key Words: Light availability, legume, nitrogen fixation, nodulation, *Pentaclethra macroloba*, *Rhizobium*

Introduction

Most legumes engage in mutualistic relationships with *Rhizobium*, a genus of nitrogen-fixing bacteria which forms nodules on the roots of their host plant. While the host plant gains a continuous supply of metabolizable nitrogen from the nodules, the *Rhizobium* obtains carbon in the form of carbohydrates from its host (Raven et al. 1971). Roughly 90% of mimosoids, 70% of caesalpinoids, and 98% of pailionoids have been found to engage in rhizobial symbioses (Allen and Allen 1981). The ability to form rhizobial associations, along with mycorrhizal associations allowing for increased mineral uptake, allows leguminous plants to grow and thrive in nutrient-poor soils. In Neotropical lowland rainforests, legumes typically constitute 12-15% of total tree flora (Rundel 1989).

Pentaclethra macroloba (Mimosaceae) is a canopy tree with *Rhizobium*-nodulated roots, dominant in the Atlantic lowland rainforests of Costa Rica (Hartshorn 1983). *Pentaclethra macroloba* tends to be abundant on poor soils but absent from richer, recent alluvial soils, apparently because it is not able to take advantage of nutrient-rich soils to the same extent as other species (Oberbauer 1983). However, Oberbauer (1983) found that in old alluvial soils, *P. macroloba* can be limited by nitrogen. Although nitrogen was sufficient for slow growth rates of fully

shaded seedlings, it was limiting for faster growth rates of seedlings growing in partial shade or full sun. Where nitrogen is limiting, rhizobial associations can be expected to increase growth rates and vigor of legumes. However, host plants growing under low light conditions in nitrogen-poor soil may be more limited by carbon than nitrogen and will therefore be unable to allocate carbohydrates to rhizobia. We can therefore expect a spectrum of nodulation (total nodule mass) across plants from different light environments with correspondingly different carbohydrate production abilities.

In this study we examine the variation in *Rhizobium* nodulation of *P. macroloba* seedlings growing under a spectrum of natural light environments. We hypothesized that seedlings growing in light gaps would show more nodulation than seedlings growing under dense canopy, and that nodulation would be correlated to canopy cover. Seedlings under dense canopy are expected to have insufficient carbohydrate production to maintain rhizobia. On the other hand, seedlings growing in light gaps are expected to have sufficient carbohydrate production to support rhizobia and would in turn require the nitrogen provided by the nodules for increased growth and metabolic rates.

Methods

This study was conducted at La Selva Biological Station, Heredia Province, Costa Rica in November 1998. Forty-six *P. maculosa* seedlings were located in a spectrum of light conditions throughout the forest. Thirty-nine of the seedlings were on residual, ultisol soils, which comprise roughly two-thirds of the La Selva forest (Parker 1994). Residual soils are highly weathered, and relatively less fertile and more acidic than the alluvial inceptisols and entisols which comprise the remaining land area. Sites on ultisol soils were chosen because nitrogen content is generally lower, and therefore potentially more limiting in these soils than in alluvial soils. The remaining seven seedlings were on old alluvial soils.

To control for plant mass, only seedlings which lacked cotyledons and were shorter than 0.65 m tall were selected. Seedlings with cotyledons still attached were rejected because seedlings can persist on the high nutrient content of the cotyledon for a significant portion of their establishment time (David Clark, pers. comm.). Seedlings taller than 0.65 m tall were rejected to minimize the confounding effects of plant mass on nodulation. Seedlings were excavated with a shovel by cutting a circle of roughly 0.5 m diameter in the soil around the seedling, then loosening the soil around the roots carefully with our fingers until the seedling came free. Although care was taken not to break any rootlets, some inevitably broke off in the process of loosening the soil. Thus values we obtained for nodulation should be regarded as minimum. Densimeter readings were collected (by the same investigator throughout the study) in four directions at the crown of each seedling, then averaged to get an estimate of canopy

coverage. Canopy coverage is assumed to be a measure of light-availability, with no coverage representing 100 % of full sun and full coverage representing 0 % of full sun.

Roots were washed in water, then examined for nodules. Nodules were counted and weighed fresh, then examined under a microscope for evidence of nitrogen-fixing activity. Nodules with pink coloration in the interior were considered active. Pink coloration indicates the presence of leghemoglobin which creates the anaerobic environment necessary for nitrogen-fixation (Verma and Stanley 1986). Inactive nodules appeared whitish on the inside. Plant heights were measured in the lab. Seedlings were weighed after drying for fifteen hours at 80° C in drying ovens.

The effects of light regime and plant mass on nodule mass were analyzed using a multiple regression with two explanatory variables. Although we tried to control for plant mass by selecting seedlings of similar size, we saw variation among seedlings. We expected variation in plant mass to be positively related to nodule mass, but it was possibly related to light regime as well. To test for non-independence of plant mass and percentage of full sun we used Pearson's correlation coefficient. We found a positive but weak relationship between light regime and plant mass ($r < 0.5$; see Results), so we included plant mass as an explanatory variable in the model. Values for percentages of full sun were natural-log transformed prior to analysis.

Soil cores were collected down to a depth of 30 cm next to each seedling and tested for phosphorous and nitrogen to account for possible effects of soil nutrients on nodulation. Soil nitrate and ammonium

are known to decrease nodule mass and nitrogen-fixation activity (Verma and Stanley 1986). Conversely, phosphate can increase nodulation as *Rhizobium* requires two to three times more phosphorous than the host root (Schenk 1986). Fifteen samples were analyzed for phosphate, ammonium, and nitrate content by colorimetric analysis described by Anderson and Ingram (1989). Ten percent of full sun was arbitrarily chosen as dividing line between high and low light regimes. All 5 samples from seedlings growing under > 10 % of full sun were analyzed, as well as 10 randomly selected samples from the seedlings growing under < 10 % of full sun. Inappropriate blanks were used which contained only KCl, so absolute soil nutrient concentrations could not be determined. Since all samples contained the same proportions of reagents, however, variation between absorbances was nevertheless assumed to be due to variation in nutrient concentration, so absorbances were recorded and compared using a Wilcoxon Rank Sum test. All statistical analyses were performed using the JMP statistical program (Sall and Lehman 1996).

Results

Rhizobium-nodules of *P. maculosa* seedlings showed the indeterminate growth patterns described for other mimosoids (Sprent et al. 1986). Small nodules examined were spherical to oblong, whereas larger nodules were highly branched, with distinct apical meristems. Most nodules were active, and no nodules weighing more than 0.45 g were inactive. No nodules were found on any seedlings growing under less than 2 % of full sun.

Light regimes ranged from 1 to 30.5 % of full sun. These values are

derived from densiometer estimations of percent canopy coverage; because these are subjective measurements, they are useful as relative, not absolute values, and may not be directly comparable to other measures of light availability. Mean (S. E.) plant mass (dry wt) was 4.16 (0.24) g. Mean (S. E.) plant height was 45.58 (1.28) cm.

The results of the multiple regression are given in Table 1. A significant amount of variation in nodule mass is explained by both light regime and plant mass ($R^2 = 0.39$, $P = 0.0006$). Use of plant mass as an explanatory variable in the model was justified by its weak correlation to % of full sun (Pearson's correlation coefficient, $r = 0.3916$, $P = 0.0078$). The positive relationship between light regime and nodule mass is shown in Fig. 1. The two outliers with high nodulation at 8.3 % and 10.7 % of full sun were larger seedlings than most others with masses of 6.9 and 6.3, respectively.

The Wilcoxon Rank Sum tests revealed no significant differences in the levels of phosphate, nitrate, and ammonium between high and low light regimes (phosphorous: $Z = 0.48$, $P = 0.6321$; nitrate: $Z = 0.69$, $P = 0.4919$; ammonium: $Z = 0.69$, $P = 0.4922$).

Discussion

To a legume, rhizobial association represents a trade-off of carbohydrates for metabolizable nitrogen. Under conditions where photosynthetic rates are high and nitrogen is limiting, it can be expected that the symbiosis will flourish, with rapid growth rates of both *Rhizobium* and host plant. High photosynthetic rates allow for increased carbohydrate production to support increased nodulation. In return, *Rhizobium* nodules allow for increased

growth rates in its host plant in a positive feedback loop. However, environmental conditions that limit photosynthesis and thereby create carbon stress in the plant, such as drought, high salinity, low temperatures, defoliation, and low light availability can be expected to limit nodulation.

Our findings are consistent with this model of *Rhizobium*-light interaction mediated through the host plant. Nodulation in naturally occurring *P. macroloba* seedlings at La Selva was found to increase with increasing light availability. Moreover, no seedlings growing under less than 2 % of full sun had nodules. Nodulation was also correlated with plant mass. This is as expected, as larger plants tend to have larger photosynthetic capacities than small plants, independent of light availability.

A possibly confounding factor in our data collection was our ignorance of the history of the light gaps in which some seedlings were collected. Root nodulation would be expected to show a delayed response to increased available light. Seedlings of similar size collected under light gaps of equal size would be expected to show a gradient of root nodulation related to the age of their respective light gaps. This pattern would help to explain the distribution of seedlings with no nodule mass across all light environments (see Fig. 1). Further study is necessary to elucidate the response rate of root nodulation to increases in light availability, such as those provided by light gaps formed from branch or tree falls.

Mycorrhizae fungi, which occur simultaneously with *Rhizobium* in most legumes, could be a further confounding factor. *Pentaclethra macroloba* has been shown to be obligately mycotrophic,

meaning that it cannot survive to reproductive maturity at naturally occurring fertility levels without mycorrhizal association (Janos 1980). Mycorrhizal infection has been shown to increase number of nodulated seedlings, as well as nodule abundance and size on many tropical legume species, including *P. macroloba* (Janos 1980). The most likely benefit rhizobia gain from mycorrhizae is increased phosphorous uptake by the plant, although other factors are implicated as nodules often respond more to mycorrhizae presence than to fertilization with phosphorous (Daft and El-Giahmi 1974).

Under certain conditions mycorrhizae can be detrimental to both host plant and *Rhizobium* by acting as a carbon drain. The carbon required by mycorrhizae and *Rhizobium* has been estimated to be around ten percent of the total photosynthate produced by the host plant (Schenck 1989). When the photosynthetic capacity of the host plant is low, mycorrhizal plants can have reduced growth relative to non-mycorrhizal plants (Furlan and Fortin 1973). It has further been shown that under conditions of carbon limitation, mycorrhizae will persist at the expense of rhizobia (Bayne et al. 1984). This suggests that in legumes engaging in both symbioses simultaneously, rhizobia are more sensitive to changes in light availability to the host plant than mycorrhizae.

In high light environments rhizobia may benefit from mycorrhizae through increased phosphorous uptake and increased plant growth. In low light environments mycorrhizae may be detrimental to rhizobia due to its superior ability to sequester carbohydrates during periods of low photosynthetic rates in the host plant. Thus mycorrhizae may increase the disparity in

nodulation between high and low light environments. This hypothesis could be tested by comparing nodulation on seedlings inoculated with *Rhizobium* and seedlings inoculated with *Rhizobium* and mycorrhizae under a range of light environments and comparing nodulation.

Pentaclethra maculosa is usually classified as a shade-tolerant species. That *P. maculosa* is capable of growing well under low light conditions is well-established (Huston 1982). However, the generalized classification becomes dubious when its growth patterns are examined in detail. *Pentaclethra maculosa* has been found to be a common constituent of successional forests of all ages at La Selva, with growth rates comparable to those of early successional species (Werner 1985). Oberbauer (1983) observed surprising plasticity in growth rates of *P. maculosa* in response to increased light intensities in controlled growth experiments. Considering the high frequency of light gaps and the heterogeneity of light environments found in the forest at La Selva (Sanford 1986), this plasticity may help explain the unusual abundance of *Pentaclethra maculosa* at this site, where it constitutes 51 % of individuals in the canopy (Lieberman and Lieberman 1987). The flexibility of the rhizobial association observed in this study is probably an important component of the ability of *P. maculosa* to maximize growth in varying light regimes.

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Table 1. Results of multiple regression using ln (% full sun) and plant mass (g dry wt) as explanatory variables for *Rhizobium* nodule mass (g fresh wt) in *Pentaclethra macroloba* seedlings at La Selva Biological Station, Costa Rica.

Term	Estimate	S. E.	<i>t</i> -ratio	<i>P</i>
Intercept	-0.27	0.07	-3.71	0.0006
Ln (% full sun)	0.09	0.04	2.67	0.0108
Plant mass	0.05	0.02	3.15	0.0030

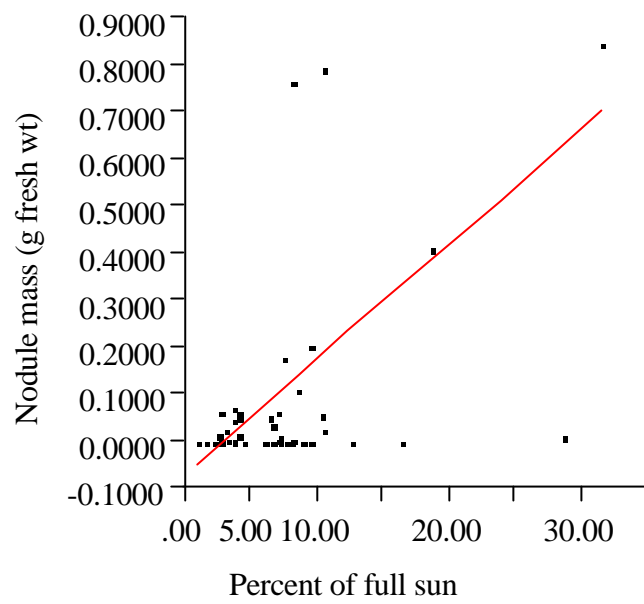


Fig. 1. Scatter plot of *Rhizobium* nodule mass as a function of percentage of full light in *Pentaclethra maculosa* seedlings at La Selva Biological Station, Costa Rica.

THE BACK OF THE BOOK

Quotes

“I’m dangerous when I’m hydrated.”

–Sophia

“Hard work sucks.”

–Jon

“Devil’s Hole pupfish beanie babies, get them before they go extinct.”

–Greg

“Yeah bring your bins, definitely bring your bins.”

–Chris

“I have a shalauqued sea turtle in my basement, I don’t know why.”

–Sophia

“Bon Jovi’s my man”

–Kristen

“I dreamt I was the kingdom Mycelium and when I tooted I knew all of the phyla because I created them.”

–Lisa

“Bust out”

–Chris

“It turns out”

–Jon

“You can be the punch and ‘other activities’ committee.”

–Chris

“Well I was looking for a congratulations sign and a bunch of chikis at the top”

–Michelle, after reaching the summit of Cerro Chai at Las Alturas

“How many of you have taken hallucinogenic mushrooms? That’s good, I take them whenever I can.”

–Luis Diego Gomez

“Man, it would suck to be a prostitute in this town.”

–Jessica

“I like the plants that make me think of sex, like lilies scream fuck me fuck me fuck me!” –

–Sophia

“I think we should all get naked.”

–Cory

“Why do insects go to flowers? Maybe for food, maybe to get laid.”

–Chris

“The little mousy ... just went down the curtain and into my bed.”

–Naamal

“The nature of our experiment was similar in nature but different.”

–Andy

“I remember shining the flashlight in Ethel’s eyes.”

–Margaret

“I’m a woman, I can do what I want.”

–Cory

“When you mix orange tang with mango tang you get orangutang”

–Sarah

1) Greg makes weird noises.

2) Serena: shut up or I’ll kick your ass!

3) Greg: Is that what they teach you to say to boys at a woman’s college?

“Maybe if we set Meffe and Carroll to music”

–Sarah remarking on her inability to remember any MC but she knows every song on the radio.

“Totally oatally.”

–Chris

“My hovercraft is full of eels”

–Carl

“Jugaron mejor, solo que perdieron más puntos”

-Oscar, comparing the USAP team’s style in the 22-0 loss against the Huertos team to our style in the 14-0 loss against the Taller team

“Don’t fuck with the duck”

-Sophia

“I’m glad I don’t have a boyfriend”

- Andy

“I followed a coati around for 45 minutes today waiting for it to poop. And it never did!”

-Serena

“That bird has issues.”

-Abbey, while watching a Blue-headed Parrot pacing back and forth along a branch at Las Cruces

“The high-light plants experienced more light than the shade plants.”

-Anonymous author

“Let’s keep ‘em out of double-digits!!”

-Greg, at half-time during the soccer game against the La Selva Taller team (half-time score 8-0; we didn’t).

You know you've been on the USAP course too long when...

- ...you dream of processed food like Kraft macaroni and cheese
- ...only getting ten mosquito bites is considered a good day
- ...you think 'ovipositor' when you see a sign reading "Ovi Ovi Restaurant"
- ...watching flowers while sitting on a cooler in the rain with an umbrella is a normal day
- ...you design and perform a dance called the "Agouti Booty"
- ...ripping off butterfly wings in the name of science is okay
- ...you note that the Creamas in the comedor taste "especially good today"
- ...after not eating beans and rice for two days, you begin to suffer withdrawal symptoms
- ...you break into a cold sweat at night after realizing you left your headlamp behind
- ...insect repellent containing less than 50 % DEET just won't cut it
- ...you diss a movie because it does not follow correct pollination biology
- ...chicky's and peanut butter is considered gourmet food
- ...you pay money to play on a N64 for an hour
- ...words such as Tukey-Kramer, ANOVA, and Wilcoxon non-parametric make sense
- ...you use "Palo Verde" as a curse word
- ...not showering and wearing the same set of clothes for five days in a row is acceptable and even normal
- ...you get tired at 9:00 pm and wake up at 6:00 am
- ...killing mosquitoes becomes your favorite pastime
- ...you match your socks according to the degree of mud coloration
- ...the vegetarian option consists of fried cheese and a funky string-bean omelette
- ...fights break out over the last scoop of ice-cream
- ...you know more about hummingbird pollination than the latest news
- ...you think Dan Janzen is wrong
- ...Meffe and Carroll becomes the bane of your existence
- ...getting access to email becomes your lifelong quest
- ...you can identify the owners of your coursemates' laundry piles

Bumps in the night at Cerro de la Muerte

by Serena Black and Jessica Lynch

Disclaimer:

This is a story that you probably don't want to hear, but we assure you it is solely for your own protection and, of course, reproductive success from an ecological perspective. We are telling you what the OTS jefes have tried to stifle with bribery and death threats. Of course, we can't reveal our sources...

Once, a long, long time ago, a group of undergraduates with OTS traveled to Cerro de la Muerte to spend a rustic week freezing at high elevation. Things were tough for them on the mountain. Many students were experiencing periodic convulsions from email withdrawal. Worse still, secret chiky stashes were being raided in the night. Cerro de la Muerte was a lonely and eerie place. Vegetation was sparse, and one young student was having a very hard time finding cute 19 year old Ticos to dance with. Limited cooking capacity meant rations were low; students were always hungry and scavenging was fierce at meal times. Reverberating across the rocky landscape were the echoes of chattering teeth and growling stomachs. Despite the miserable conditions, students were devoted to the pursuit of biological knowledge- never hesitating to ask questions and lengthen lecture times.

The morning of the second day dawned bright with not a cloud in the sky. The students knew of course that this did not mean that it would not rain. After breakfast the group set off, adorned in high-tech hiking gear for a plant identification walk that they expected would take them no more than 50 feet from the building. As they set out, a few students noticed a strange set of footprints- hoofed like a peccary but abnormally large... Being new to the paramo, they dismissed it as normal and thought nothing more. A little farther down the trail they noticed a crumpled chiky wrapper stuffed under a rock. They removed the wrapper and stashed it for proper disposal. The hike and the rest of the day went as planned. Nothing unusual occurred. Late that night however, one of the students was awakened by a strange noise. A slow dragging followed by scraping against wood. The moon outside the window was almost full. A pale light spilled across the floor elongating shadows and deepening the blackness. Squinting, the student peered into the depths of the room. She didn't want to turn on her flashlight for fear of insect bombardment so when she didn't see anything right away she gave up and went back to sleep.

The next morning more chikys were missing than ever before. Everyone was in an uproar and everyone was innocent. Lacking sufficient forensic evidence, they were forced to give up and go about their day. That night they set up a watch. Students switched off every two hours. It was nearly four in the morning and one of the students, one of the few young men in the group, had volunteered for the next shift. He struggled awake despite the fact that his alarm had gone off five times. The effects of the last night's rum were fogging his senses. He stumbled through the cold to the bathroom and nudged the door which creaked slowly open. It

took him a moment to realize that he was not alone. As soon as he did he was jerked awake, finding himself confronting something not of this world. It was huge and hulking. In the faint light its features were blurred. It stood in front of the toilet doing something undeterminable. It turned gleaming reddish eyes and gave him a piercing look that lasted only seconds before it leapt into the toilet and swirled away, a long scaly tail slithering behind it. (This gave the toilet monster a whole new meaning!) The student was paralyzed with fear and still standing frozen in the doorway when his impatient shift mate discovered him. Everyone was awakened and an account of the experience was given but the details were so vague that many did not believe him and some secretly suspected that it was a chiky cover-up.

The next day the students were in such desperate need of entertainment – because chess is only a two person game – that everyone joined in, even the disbelievers, to design a trap. They deliberated all day. Many people had many different ideas and consensus within the group was difficult to reach. Finally, they settled on building an elaborate structure of butterfly nets and heavy bird books. The whole thing was hung precariously above a stash of chikys – the perfect bait. A problem arose when it came time to donate chikys but in the end everyone contributed with only minor dissension. Night fell slowly with the mounting anticipation. The students were too nervous or excited to study or even play guitar. They were however, able to drink cervezas without reservation. People went to bed early but were unable to sleep. The full moon drifted slowly across the sky. Most everyone was awake when it happened. The noise has been described as a crash followed by a thud and a moaning-screeching noise that struck terror into the heart of even the bravest OTS students. They rushed into the kitchen and what they saw there changed each and every one of them forever. They all agree that it was huge, but after that the details become foggy. Some people saw wings covered with scaly feathers somewhat like the BFF spotted fleetingly on Cerro Chai. Other saw antennae- feathered and huge; they were reminded of descriptions of the mythical both. Others still saw a forked tongue and smooth chest somewhat resembling the elusive bushmaster. One girl observed muscular legs with hoofed feet and a snout like a peccary. A long slinky tail and furry head resembling the endemic toilet mouse found at Las Cruces have also been described in later accounts.

Before anyone could act, the creature tore through the nets and sprung from the trap. It fled from the cabin, melting into the night- the chikys were gone. It has never been identified, but local people tell the stories of a powerful beast that roams the paramo late at night, and occasionally a strange hoof-print is found.

Recuerdos

La Selva part I

- Rafting down the Sarapiquí
- Swimming with the caiman
- The fruit lab: pejibaye with mayonnaise- this is fruit?
- Spanish interviews with CPH
- The terciopelo on the tour. Ummm... are they all that big?

Santa Ana and CPH

- El Bosque
- Waiting for bagels
- Birding in a coffee farm
- Walking 1/2 hour to class
- Volcán Poás
- Dancing in the aisles of the bus on the way back from the beach
- Birding in knee-deep water at Carara
- Forced to dance at CPH
- Jen wins the dance contest
- Buying the course guitar

Las Cruces

- So those electric shower units really do work .
- So those electric shower units really can shock you.
- The garden tour that never toured
- Everything you ever wanted to know about CAM photosynthesis and so much more.
- “Are there any native plants in this garden?”
- Raul’s Toucan obsession
- “Can the chef travel with the course?”
- Luis Diego’s amazing botanical pharmacy
- Email troubles, take one
- The Both
- Beginning to recognize everyone’s underwear
- Casa Wilson gymnasium
- The “X-files” (What’s with the corn?)
- Discomoviles
- The talent show
- The significantly uneven distribution of insect bites on Sophia’s legs
- Plant walks
- Falling in the water downstream from the matadero
- Carl comes out of the closet about his juggling skills

Las Alturas

- The “cooked” spaghetti
- Eduardo in the toyotona
- There’s a bushmaster out there somewhere
- Hiking in the rain.
- Sliding downhill in the rain
- Chips and guacamole and studying
- Backrub chairs and sleeping on the porch
- The hammock

Cerro de la Muerte

- Darren in his sleeping bag
- Marteen’s “Gentry transects”
- Only the foolish brave the showers
- Computers available only when the generator’s running
- Chris and Erika buy Chickeys
- Huddling next to the fire: we weren’t warned that it would be this cold!
- Are these guys ticos? Where’re the beans?
- Research seminars with Maarten: “Okay, very good, sit down.”
- The perfect morning on the paramo
- Looking for quetzals in the rain at 5:30 am

Palo Verde

- The mosquitoes.
- The rain
- The faculty insisting that the mosquitos are “not so bad.”
- More mosquitoes
- More rain
- Margaret’s boot disappears into the Mangrove Mud . Andy heroically rescues it.
- Carl stays clean and pressed in the mangroves. How did he do that?
- Ice Cream
- Wondering if the laundry will ever return
- Looking for your matching sock when it did
- The crema-chickey debate begins
- Hiking through crocodile infested waters (i.e. the road)
- Carlos driving on the flooded road with everyone packed into the car.

Rincon de la Vieja

- The raw meatballs
- “How can you be lost if you’re at a ranger station?”

- “Please, laundry only from those who are really dirty.”
- Cory takes a facial mud bath

La Selva part II

- Everyone becomes a vegetarian
- “Ask Orlando, he’ll know”
- USAP- 0, Taller - 14
- Pizza and video night
- Invaded by Carleton College
- Still more rain
- Email troubles take two: the server’s down!
- Suddenly everyone wants to see a terciopelo
- The banana plantation
- the Salon in La Guaria
- Hummingbird foraging behavior, in the rain
- Is this the dry season?
- Chickeys thrown over for cremas
- USAP – 0, Huertos – 22
- The interlink bill arrives
- Erika’s voodoo frogs
- Playa Cahuita

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