

***PSE&G ENVIRONMENTAL EDUCATION GRANTS
PROJECTS FOR YOUR CLASSROOM***

**Project Title: Ecology and Genetics of Leaf Gallmakers on
Goldenrod (*Solidago altissima*)**

PSE&G Grant Year: 2006-2007

Grade Levels: 10 & 12

Number of students involved:

50 students

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Project Summary

Overall purpose of project/project objectives

This initiative was designed to provide a collaborative learning, hands-on, project intended to explore ecological organization using classic ecological and molecular biology techniques. The objectives of the study were to create an opportunity for students to discover the role of plant species in organizing ecological communities; estimate species diversity, examine intraspecific and interspecific species interactions, and ecological succession; utilize modern molecular biology techniques to evaluate the genetic relationship among the leaf galls produced by the midges of the genus *Asteromyia*; and serve as a forum for investigation of various topics associated species formation and community assemblage and consider habitat fragmentation and destruction that is a result of current land use patterns on a local and global basis.

Classroom organization

From November 2006 through Oct 2008, students registered in my Environmental Science and Science Research (I – III) classes were involved in field trips and class work with field collections and some individuals worked independently on this project outside of class.

Student Research

Student research included field investigations, data mining and genetic analysis. Field studies involved census of populations of gallmakers and host plant species,

recording abiotic factors such as soil moisture and light intensities, and identification of organisms with the goldenrod community at the Great Swamp and at the Scherman-Hoffman Wildlife Sanctuary.

To address the genetic differences among sibling species, students utilized the research tools available through the National Center for Biological Information (NCBI) to find current data and recent publications on *Asteromyia*. It was found that a number of DNA sequence submissions of the cytochrome oxidase I gene have now been entered into the database and that primers for the polymerase chain reaction technique had been published. Students communicated with the published author receiving manuscripts and encouragement. Custom PCR primers were synthesized and a thermo cycler was borrowed from Rutgers University which provided an opportunity for students to use an additional investigative technique that had not previously been used in our school.

Students were also introduced to bioinformatics, using GenBank to mine for data from the mosquito genome (represents the closest phylogenetic relative to *Asteromyia* published to date) and they used that information to develop analytical tools for investigating the genetic relationship among gallmakers. Students developed restriction fragment assays for *Asteromyia* using the mosquito mitochondrial genome (mtDNA) and New England Biolab's NEBcutter V2.0 software that allowed students to simulate single and double digest restriction fragment assays. Students analyzed these results and selected enzymes to actually use in the lab to visualize differences in the number and length of fragments produced when tested on the various sibling species.

Hands-on Activities

These activities included bioinformatics/computer work to research current literature, relevant submissions of genetic data to NCBI, PCR primer design and fragment analysis assay development. Six restriction fragment assays were developed. Other hands-on activities in the classroom included identification of gall types using field collections. Gallmakers were dissected from their galls using the digital dissection microscope, dispensed into label tubes, recorded in a ledger and preserved by freezing for eventual genetic assay. More than 100 gallmakers were isolated for genetic analysis. Isolation of mtDNA from frozen gallmakers using two methods, column prep and boil prep, amplification of mtDNA using TempliPhi, via the rolling circle amplification technique, and PCR amplification of the cytochrome oxidase I gene, gel preparation and gel electrophoresis and fragment analysis. DNA from fifty individual gallmakers was extracted, amplified, restricted and analyzed by gel electrophoresis. The results of these assays are inconclusive as the method of mtDNA isolation did not produce sufficiently pure samples of mtDNA.

Impact on Students

The students involved in this project had an unprecedented opportunity to engage in authentic research in a high school environment. They were able to learn about and use techniques that they may not be exposed to again until they become upperclassmen in college.

Perhaps the most difficult aspect for the students was engaging in field work, but this is also the most important from my perspective. I think they students would not have noticed or valued the diversity of life that is all around us without this experience. This impact is measureable to the extent that four students have embarked on studies in the area of pollution remediation by plants, aquatic community succession, and environmental microbiology and one will continue to work on the *Asteromyia* system.

Unexpected impacts include some very good discussions about career and career development, especially with students in my Fall 2007 environmental science class. These students were inspired to seek out grant applications and learn more about the process, which lead to a discussion of the importance of grant writing and how grant writing is involved in an academic career.

Links to Science, math and Technology

By asking a simple question about the ecological nature of the relationship among gallmakers found on goldenrod species, students explored many aspects of science including genetics, evolution, ecology, physics, and molecular biology. Studying the diversity of organisms at the Great Swamp and Scherman-Hoffman Wildlife Sanctuary students employed the Simpson Index to calculate biological diversity. They also engaged in computational biology in the course of selecting restriction enzymes and developing assays as they gained insight into the probability of restriction enzymes recognizing specific DNA sequence patterns. This project also provided an opportunity to introduce students to the use bioinformatics and bioinformatics tools to access database information and to take advantage of this burgeoning career field should their interests take them in that direction.

Community Involvement

A newsletter article announcing PSE&G support for this project was sent to every student household in the school district. There was a lot of excitement in the community. An online photo album was developed to share our enthusiasm of the project and to acknowledge our sponsors. In addition following the Great Swamp field trip photos were posted for parents and the school community.

A summary of the project will be published in the December 2008 newsletter and students will be urged to become involved in this ongoing project and asking them to consider registering for the three-year science research program my school offers.

Other community impacts include bringing these gallmakers to the attention of the naturalists at The Great Swamp and at the Scherman-Hoffman Wildlife Sanctuary and making them aware of the ongoing basic research that is being done by professional scientists and perhaps, encouraging consideration of this unknown and overlooked system in their programs. I suspect we added to the species lists at these locations.

Materials and Budget

Material/Item	Cost
Fisher BioReagents Cool Pak Portable Coole	85.00
Fisher variable micropipets (0.5 - 10 ul)	192.55
Fisher variable micropipets (10 - 100 ul)	192.55
Fisher variable micropipets (100 - 1000 ul)	192.55
SK micropipet 2 - 20ul	184.00
SK micropipet 20 - 200ul X 2	368.00
SK micropipet 200 - 1000ul	184.00
SK Micropipet tips 2 - 20ul x 2	30.00
Caroline Pipettors 2.0 to 20 ul WF21-4653	189.00
Student Micropipets WF-21-4610	50.00
Storage Boxes	20.85
SK Digital Dissecting Microscope	699.00
Electrophoresis Lab system & extra comb	805.00
Polypropylene Micro Test Tube Rack WF21-5570	73.50
PVC pipe and connectors	50.00
Reagents:	
TempliPhi	200.00
Restriction Enzymes	354.00
1Kb Ladder	188.00
PCR Beads	149.00
Custom Primers	50.00
Mini-Prep Kit	64.00
SybrSafe Dye for Visualization of Gels	94.00
Field Trips	
Fall 2007 Great Swamp	385.00
Fall 2008 Scherman-Hoffman Wildlife Sanctuary	320.00
Total expenses:	5120.00

Evaluation

The most of the students that were involved in this project are registered in our science research program and received intensive classroom and field instruction in basic ecology in our school and through Rider University. Their knowledge of basic ecological knowledge was evaluated by writing an authentic research paper (NJCCS 5.3, 5.5, 5.8) at the beginning of this study that was evaluated by Rider University and me. Student posters were presented and defended orally (NJCSS 3.3) at the Woodbridge District Science Symposium and at the JFK Memorial High School Senior Science Symposium in May 2008. Work on this project completed since February 2008, will be presented in May 2009 at these same symposia. Students who have worked on this project on an individual basis have been guided on a daily basis and evaluated on a bi-weekly basis through reports and lab notebook checks.

Publicity/Resource Information

The proposed budget was exceeded and those costs were covered by additional grants and awards including a \$1000 award from Environmental Awareness Contest sponsored by the Middlesex County Division of Solid Waste Management that allowed for the purchase of storage boxes, tube rack, tips and micropipettes, and by a generous

donation of restriction enzymes from New England BioLabs, Inc. and their public education assistance program. I also gratefully acknowledge the assistance of the Waksman Student Scholars Program at Rutgers University who loan some equipment to our school so that we can pursue molecular biology research projects.