

**Final Report for Summer 2009 STEP-EUR sub-grants
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Title: Gene discovery in the parasitic plant *Cuscuta pentagona*: Gene cloning and characterization of expressed sequence tags.

Objectives: The five-week course served as an introduction to working in a molecular physiology lab. Students learned basic molecular biology skills, beginning with accurate pipetting, and advancing to polymerase chain reaction (PCR), DNA purification, gel electrophoresis, molecular cloning using bacterial vectors, and molecular mapping and analysis of isolated gene fragments. In addition, students were introduced to the use of computer genetic databases in developing a strategy for gene discovery, and the use of molecular biology software tools for the analysis of data generated following gene isolation and sequencing. The specific research objective for the session was to have students identify genes expressed in parasitic tissues (stem haustoria) of *Cuscuta pentagona* and to subsequently identify cognate genomic sequences in both *C. pentagona* and in its non-parasitic relative, ivy-leaf morning glory (*Ipomoea hederacea*).

Conduct of class/research: Students enrolled for three credit hours of Biol 4050 Supervised Research. The class met Tuesday through Friday for three hours per day (8:30-11:30 am) for five weeks, for a total of 60 scheduled lab hours. Grading was based on participation. Students worked in pairs. On some occasions, the class was able to dismiss early, but on most days at least some of the groups needed the entire class period, and occasionally we worked for as long as three and a half hours. The first week of class was dedicated to introducing the students to the basics of working in a molecular physiology lab, and to the background and strategy of the investigation. During the four subsequent weeks, the students were required to become increasingly independent in setting up and executing their projects.

Students: Of ten students committing to participate, nine ultimately enrolled for credit. As in past years, students participating in this research were recruited from spring semester, 2008, Biol 1450 and 1750 classes. These courses are the two introductory-level courses, botany and zoology, required of all UNO biology majors, and it is presumed that the students in these courses are in the earlier part of their biology degree. Tuition payment was made available only to students with freshman or sophomore status. Current UNO biology students enrolled in the summer 2009 research session were Adam Anderson, Alek Erickson, Jason Brisbin, and Mengyi Zha.

Pre-freshmen were recruited from the pool of dual enrollment (DE) and advanced placement (AP) high school science students from Omaha metropolitan area. Students recruited from the local high school DE/AP student pool were, by school, Mona Baishya and Mollie Hippensteel of Millard North High School, Amanda McIntyre of Marian High School, Callie Tometich of Ralston, and Laine Anderson of Bellevue West High School.

In addition to nine students enrolled for credit, one local home-schooled student, Patricia Hanus of Ralston, assisted in the research without credit.

Technical and research assistants: Jacklyn Miller, an incoming UNO undergraduate (beginning Fall 2009) and participant in the 2008 STEP summer program, assisted with the summer 2009 session. Danivia Bustamante, a biotechnology undergraduate of UNO, also assisted in running the laboratory as an intern.

Both Jacklyn and Danivia are continuing to work on the project through the remainder of the summer session.

Research accomplished: Each student researcher was provided with a unique collection of 40 unknown expressed sequence tags (ESTs) generated from stem or parasitizing haustoria of *C. pentagona*. The ESTs were maintained in plasmid cloning vectors in *E. coli* colonies. Students performed a size screen PCR amplification to generate DNA fragments for size comparisons, and on the basis of size, chose two or three distinct clones for sequence analysis. A total of 24 ESTs were analyzed by sequencing, and each student had the opportunity to work with the DNA sequencer twice. Sequence data generated by students was used to search available gene databases to provide putative identities for the cognate genes.

Several genes were putatively identified to a high degree of confidence: among these, a COP1-like ubiquitin ligase (important for its role in photomorphogenesis), sucrose phosphate synthase (cognate to a genomic sequence identified in a previous session) and an inorganic pyrophosphatase. From these sequences, the putative ubiquitin ligase was chosen for further characterization from the *C. pentagona* and *I. hederacea* genomes. Using the student-generated sequence data and additional database sequences, oligonucleotide primers were designed for the attempted cloning of these gene fragments from dodder and morning glory. With genomic DNA isolated in class, the students were able to amplify candidate fragments for sequence analysis. This final experiment marked the end of the five week session, and consequently, analysis of these putative dodder and morning glory gene fragments is ongoing.

Results from previous years' efforts were reported in posters presented at the Ecological Genomics meeting hosted by Kansas State University in Kansas City, MO, in November 2008 and at the UNO Undergraduate Research and Creative Activity Fair in the Spring of 2009, for which Sydney Brommer, my former laboratory assistant, received third prize among undergraduate research posters.

Ongoing research with STEP program students and other undergraduates: Currently there are three undergraduate researchers working in my lab on the *Cuscuta* gene project. In addition to Danivia Bustamante and Jacklyn Miller, Branden Poe, a participant of the 2008 summer research program, has continued, and was awarded a UCRC grant in the Fall of 2008 to support his work.



Summer 2009 participants, L-R, front: Jacklyn Miller (laboratory assistant); second row: Zed Zha, Callie Tometich, Danivia Bustamante (biotechnology intern), Mark Schoenbeck (instructor); third row: Patricia Hanus, Laine Anderson, Mollie Hippensteel, Mona Baishya; back row: Jason Brisbin, Amanda McIntyre, Adam Anderson, Alek Erickson.