CALIFORNIA STATE SCIENCE FAIR
2009 PROJECT SUMMARY

Name(s)
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Project Number
J0424

Project Title
Next of Kin: Comparing Nucleotide Sequences of Native Plants

Abstract

Objectives/Goals
The purpose of this project was to find differences in the nucleotide sequences of a highly conserved chloroplast gene of different native plant species. This involved extracting DNA from native plants, and amplifying using polymerase chain reaction (PCR) and then sequencing the gene that codes for the enzyme RuBisCo (Ribulose 1.5 Bisphosphate Carboxylase/Oxygenase). This data was included with other native plant sequences available on GenBank to test the hypothesis that the unique and essential RuBisCo gene nucleotide sequence would be more similar between two species of native ferns (Pteridophyta) than that of flowering plants (angiosperms). This is expected because ferns evolved about 385 million years ago, that is 250 million years before flowering plants.

Methods/Materials
DNA was extracted from plant leaves using different methods: leaf grinding alone; adding sand during grinding; and using the Qiagen DNEasy protocol (QDEP). Extracted DNA was amplified by PCR and run through gel electrophoresis. Successful samples were purified using Qiagen QIAquick protocol, followed by gel electrophoresis and spectrophotometer readings. PCR was rerun to increase concentrations and samples were sent to SDSU for sequencing. These two nucleotide sequences were included with sequences of other native species downloaded from GenBank to run summary statistics. All data were input to Blast and Clustal W2 software to produce a phylogram of relationships.

Results
Data from GenBank showed that the two fern species compared --P. scoulari (leather fern) and P. glycorrhyza (licorice fern) # were most similar with only 11 differences of the 1322 base pairs (99.17% similar). Lupinus albifrons (silver bush lupine) was most different from the ferns with 254 and 258 nucleotide base differences respectively. PCR was successful only on two of the six plants extracted using the QDEP: Erysimum concinnum (curly wallflower, a rare plant) and Lupinus rivularus (streambank lupine).

Conclusions/Discussion
The resulting phylogram of all data shows a more recent evolution in flowering plants than in fern species consistent with the hypothesis. The success of the QDEP is likely due to the fact that it removes polysaccharides that interfere with PCR. The successful samples were from younger leaves, which may have improved DNA extraction. The two successful PCR sequences are being prepared for submission to GenBank.

Summary Statement
The purpose of this project is to compare nucleotide sequences of a unique and essential chloroplast gene in different native plant species and to contribute new local and rare plant sequence data to GenBank.

Help Received
I was mentored by Dr. Jianmin Zhong who provided his laboratory at Humboldt State University. I also consulted with Dr. Jacob Varkey and Anthony Baker. My mother learned along with me and my parents criticized my writing. Botanists Laura Julian and David Imper helped me identify native plants.