The Evolutionary and Ecological Roles of Illinois Listed Endangered and Threatened Grasses

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Specific Aims

This research will investigate the genetic evolution of endangered and threatened (E/T) grass species listed in the state of Illinois to help reveal the genetic diversity these species add to the Illinois flora. The specific aims needed to achieve these goals are to first sequence the DNA of the plastid genome (plastome) for each of the E/T grasses; this DNA contains the species' unique genetic information. Next these data will be used to determine the evolutionary history of these grasses and the changes in their DNA over time. From this history, the relationships between these E/T grasses can be elucidated as well as their relationships to other members of the grass family, Poaceae. Then, these relationships will be evaluated in the ecological context of Illinois grasslands to understand the genetically-determined roles of these species in the biodiversity of Illinois, i.e., the number of species, and the variety of their genetic traits, found in the areas they inhabit. This line of research will reveal the uniqueness of these grass species and the positions they fill in their environment.

Preliminary Results of Endangered/Threatened Species Source Collections to High Throughput Sequencing

Species	Source*	Year Collected	DNA (ng/uL)	# of Reads (M)
Beckmannia syzigachne	GRIN	2013	18.7	1.15
Calamagrostis porteri subsp. *insperata*	ILLS	2013	54.5	18.8
Deschampsia flexuosa	ILLS	1997	80.2	21.4
Dichanthelium boreale	ILLS	2009	29.7	19.9
Dichanthelium commutatum	EIU	1973	17.6	17.7
Dichanthelium portoricense	MICH	1998	30.7	TBD
Dichanthelium ravenelii	ILL	1973	6.8	2.9
Dichanthelium dichotomum	EIU	1999	16.5	17.5
Elymus trachycaulus	DEK	1987	22.1	11.0
Glyceria septentrionalis var. *arkansana*	ILLS	2001	25.9	25.5
Poa alsodes	ILLS	2003	15.6	12.4
Poa languida	DEK	1941	13.5	1.9
Poa wolfii	ILLS	1998	12.6	11.7
Schizachne purpurascens	ILLS	2013	10.1	19.0

*DEK = Northern Illinois University Herbarium, EIU = Stover-Ebinger Herbarium, GRIN = Germplasm Resource Information Network, ILL = University of Illinois Herbarium, ILLS = Illinois Natural History Survey, MICH = University of Michigan Herbarium.

Research Plan

Using the phylogenetic information learned from the NGS, phylogenetic diversity (PD) will be examined. Evolutionary trees will be constructed with the ~140 grass species native to Ilinois, including, and then excluding, the 18 E/T species. The PD will be assessed for each tree in two ways following the methods of Barber et al. (2017). The differences between these measurements will be a good indicator of the role the E/T grasses hold in the PD of the Illinois ecosystems. Tracing the PD of the endangered species in this study to the subfamily and tribe designations will provide insights into the evolution of these individuals and the niches that they fill in their environment. The results of these investigations will demonstrate the degree to which the E/T grasses contribute unique and irreplaceable qualities of the PD of the Illinois grass community. This information will provide a better understanding of the importance of these endangered species in their environment, determine the role of these species in their habitats, and what ecosystem services may be damaged or lost should one or more of the species be allowed to become extinct.

Introduction

The Illinois Endangered Species Protection Board (IESPB) has listed 251 plant species as endangered and 73 plant species as threatened (IESPB 2015). Of these listed plants, 18 are grasses, 17 endangered and one threatened (IESPB 2015). The family of grasses, a group of over 12,000 species, includes such nutritionally important crops as corn, wheat, rice, oats, barley, sorghum, sugarcane, and millet. People also use grasses as food for livestock, ornamentals for landscaping, and turf grasses for sport activities. Products made from grasses include paper, fabric, and construction materials. Grasses also provide protection from soil erosion (Judd et al. 2008). The conservation of grass species is important to maintain the continued benefits people receive from these essential plants and the biodiversity these grasses contribute to the ecosystems of Illinois. This study will evaluate the evolutionary histories and phylogenetic diversity of these E/T grasses.

Methods

These endangered grasses will be studied by sequencing the DNA of their plastid genomes to establish robust evolutionary relationships to other grasses. Next generation sequencing (NGS) techniques will be used to assemble full plastid genomes (Wysocki et al. 2014). Four of the 18 E/T species have already been sequenced and published (Saarela et al. 2015, Duvall et al. 2016). Leaf material for the remaining 14 endangered species has been obtained with permission from preserved herbarium specimens for DNA testing. I successfully modified the DNA extraction procedure to recover DNA from these, sometimes decades-old herbarium specimens from known localities (unpublished results). This avoids the permits that would be needed to collect from live, E/T plants. I homogenized leaf tissue in liquid nitrogen and extracted DNA using the DNeasy Plant Mini kit (Qiagen, Valencia CA, USA). I modified the manufacturer's protocol by increasing the starting material and made some other changes to the procedure as suggested in Drábková et al. (2002). The plastomes of the fourteen endangered grass species will be determined following procedures similar to those used by Burke et al. (2016). From these DNAs, DNA libraries will be prepared. The libraries will be sequenced on an Illumina HiSEq 3500 instrument at the Core DNA facility at Iowa State University (Ames, Iowa, USA). From the resulting DNA sequences, plastomes will be assembled according to the methods of Wysocki et al. (2014). The plastomes will then be subjected to analyses to place them into a phylogenetic tree of Poaceae using Bayesian and Maximum Likelihood methods. From this analysis, the evolutionary history of these endangered species and how they relate to one another and the other species in the family will be determined.

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Acknowledgments

Thank you to Sean V. Burke for his work with the DNA library and the herbariums listed for the plant material used in the study. This work is supported by grants from the Elwood and Ruth Briles Memorial Fund and the Illinois Association of Environmental Professionals.