



Impacts of Location and Pollinator Host Plant Presence on Genetic Diversity

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Introduction

The Eastern Prairie Fringed Orchid

(*Platanthera leucophaea*) is a federally threatened orchid native to midwestern wet prairies. Once a widespread species, it has been declining due to native habitat alteration and fragmentation. Studies of some small, fragmented populations have found high levels of inbreeding and low levels of genetic diversity. Inbreeding depression may result from elevated levels of inbreeding in these smaller populations.



Fig. 2. Platanthera leucophaea

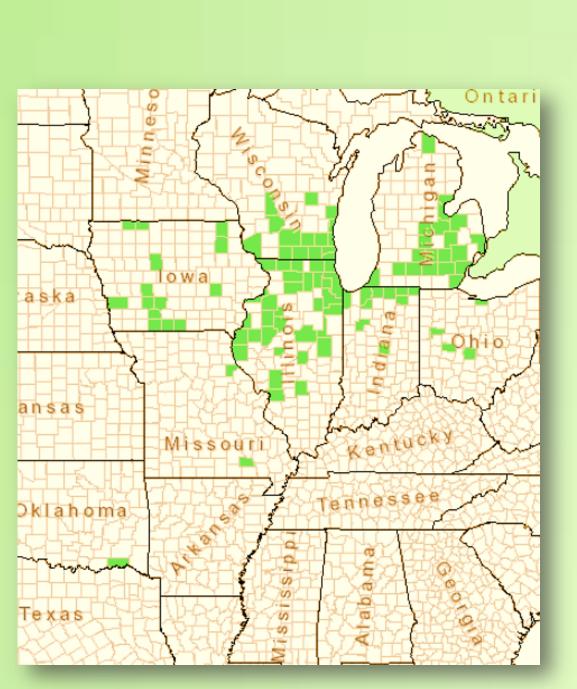


Fig. 1. P. Leucophaea range– USDA Plants Database

Pollinator limitation may be a cause of increased inbreeding, which can arise from a lack of larval host plants of the orchid's pollinator, the hawkmoth. No range-wide genetic studies have been conducted on this species, hence a more comprehensive genetic study will allow us to better detect and mediate the effects of low genetic diversity, including increased susceptibility to disturbance and risk of extinction.

In this study, we aim to assess the genetic diversity between four rangewide *P. leucophaea* populations as well as the genetic diversity and morphological fitness of five Illinois populations with varying amounts of surrounding larval host plants.

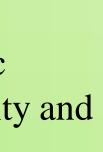
Hypothesis:

- The populations far from the center of the range will have lower genetic diversity, as populations at the edge are more distant to center of diversity and experience reduced gene flow
- There will be a positive relationship between larval host plant abundance and orchid fitness, as more host plants will result in the presence of pollinators from different populations.



Restoring *Platanthera Leucophaea*:

Methods

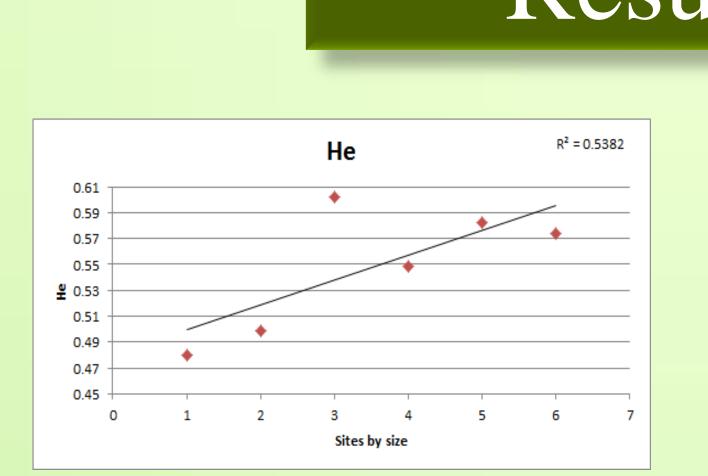


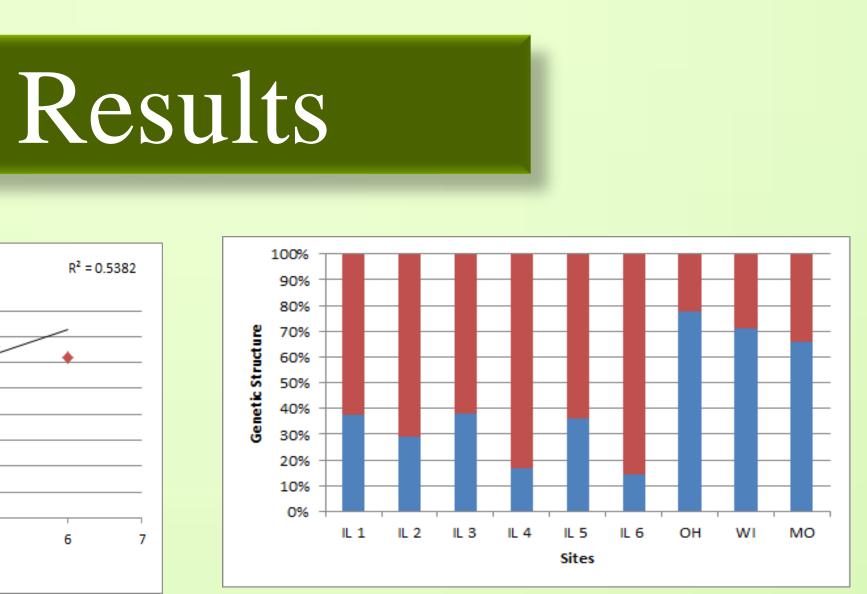
- **DNA isolation:** DNA was extracted using a CTAB-based protocol with leaves from 34 sites
- Host plant surveys: Potential hawkmoth host plant abundance was determined at 10 sites in 1m by 1m quadrats at the edges of each population. Morphological data from 2014 was also used as a measure of fitness.

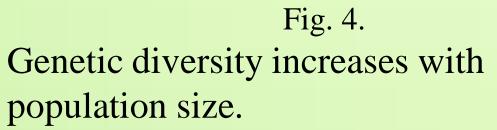


Fig. 3. Host plant surveys

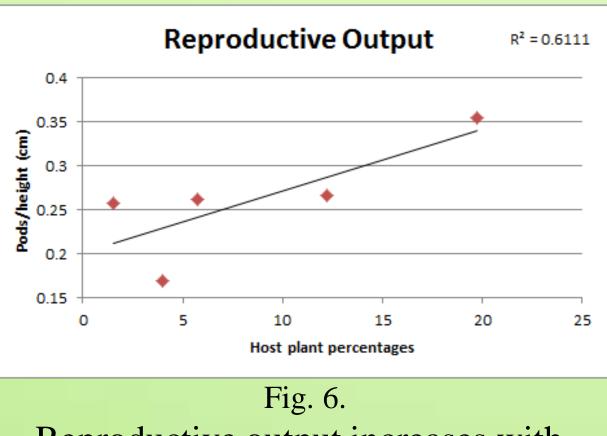
Genetic analysis: We did PCR analyses following standard protocol using 8 pairs of microsatellite primers on our extracted DNA. To genotype each individual, we used the Beckman Coulter CEQ 800 Genetic Analysis System. Data was analyzed using GenAlEx and Structure.

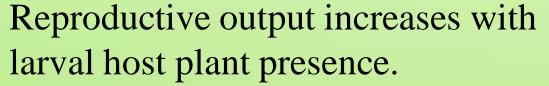






Structure analysis reveals the Illinois





600 500 端 400

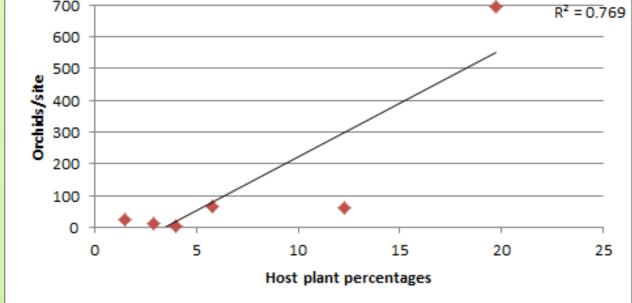


Fig. 7. Population size increases with larval host plant density.



Fig 5. populations are only slightly more related to each other than to populations of other states.



Conclusion

All nine sites across our studied portion of the *P. leucophaea* range demonstrate significant signs of inbreeding, and a decrease of genetic diversity with a decrease in size (Fig. 4). This demonstrates that inbreeding is occurring at high levels range-wide and that small populations are also subject to lower levels of genetic diversity. However, little relationship is evident between geographic and genetic distance, as Figure 5 demonstrates. This can imply that historically, the populations across the *P. leucophaea* range were well connected by pollinators. Hence, pollinator limitation could be a more recent problem that has not yet strongly affected the genetic makeup of orchids range-wide. Future study of more recent detriments to the hawkmoth habitat and more comprehensive range-wide P. leucophaea assessment could Fig. 8. P. leucophaea restoration therefore be beneficial.



No strong relationship is evident between inbreeding and host plant presence, but this is not the case between reproductive output or population size and host plant presence (Figs. 6 & 7). These results suggest that factors associated with healthy communities such as high host plant density and large population size are positively correlated with morphological fitness measures and reproductive output. These findings will inform future P. leucophaea restoration planning by emphasizing the importance of larval host plant presence and surrounding habitat management for the persistence of the orchid.

References

Pollack, C. (2009) Restoration of the Eastern Prairie Fringed Orchid (Platanthera leucophaea): Natural Pollinators and the Abundance of Larval Host Plants (Master's thesis). Northeastern Illinois University, Chicago, IL. 62.

Ross, A., Aldrich-Wolfe, L., Lance, S., Glenn, T. & Travers, S. E. (2013) Microsatellite markers in the western prairie fringed orchid, Platanthera praeclara (Orchidaceae). Appl. Plant Sci. 1: 1-4 USDA, NRCS. 2015. The PLANTS Database (ht plants.usda.gov, 11 August 2015). National Plant Data Team, Greensboro, NC 27401-4901 USA

Wallace, L. E. 2002. Examining the effects of fragmentation on genetic variation in Platanthera leucophaea (Orchidaceae): Inferences from allozyme and random amplified polymorphic DNA markers. Plant Species Biol. 17: 37–49

Acknowledgements

I would like to thank NSF-REU grant DBI-1461007 for support. In addition, Thank you to Oakton Community College for funding and the U.S. Fish and Wildlife Service for leaf samples. Finally, thank you to my mentors, Claire Ellwanger and Jeremie Fant for their guidance, and my partner Rachel Wells for her teamwork and data analysis.



Fig. 9. P. leucophaea hand pollination

