Title of Project: Pollen Limitation of Reproduction in *Dichantheliium leibergii*

Proposed by: Maria Wang

Pollen limitation occurs when insufficient quantity or quality of pollen reduces plant reproductive success[1], which can greatly impact the growth and persistence of plant populations. *Dichanthelium leibergii*, an important native prairie grass whose habitat is highly fragmented, faces a risk of reproductive failure and eventual local extinction due to pollen limitation. To determine the extent to which reproduction of *D. leibergii* is pollen-limited, I will carry out a pollen supplementation and exclusion experiment in two populations of *D. leibergii* – one with high numbers of *D. leibergii* plants, and the other with fewer plants. The findings from this study will provide valuable information for the conservation of *D. leibergii*, as well as contribute to a better understanding of pollen limitation in wind-pollinated plants.

Pollen limitation is caused by inefficient pollen transfer or a lack of pollen donors, compatible pollen, or pollinator visitations in animal pollinated plants[1]. Pollen limitation lowers chances of successful fertilization in plant populations, which hampers their seed production, potentially affecting their entire life cycle[1]. Low seed set (i.e. low proportion of viable seeds) produced by pollen-limited plants may thus lead to a decrease in population growth rate. *D. leibergii* is a species of conservation concern, because it tends to grow only in high quality prairies, making it a good indicator of prairie ecosystem health[2]. Due to habitat fragmentation, *D. leibergii* populations are rare and isolated. Germination rates are low for this species[3], which may reflect low seed set in fragmented populations. This is alarming because poor reproduction leads to population declines and eventual local extinction of the species. I suspect that pollen limitation may be the cause of low seed set and germination in wind-pollinated *D. leibergii*. Hence, investigating pollen limitation will provide valuable insight into the dynamics of habitat fragmentation on *D. leibergii* populations, which will greatly aid conservation efforts for this important prairie species.

It has been suggested that wind pollinated species are typically not expected to be pollen-limited[4]. For example, only one of ten wind-pollinated species (comprising sedges and forbs) studied by Friedman and Barrett (2009) was found to be pollen-limited[4]. However, other studies found that pollen dispersal limits reproduction in wind-pollinated trees[5,6]. It should be noted that these species are not grasses, so the results may not apply to *D. leibergii*. Pollen limitation has been reported in three species of marsh grasses[7]. Because concentrations of wind-dispersed pollen decrease sharply with increasing distance from the source[9], plants located in small or sparsely populated patches tend to receive less pollen than plants with many close neighbors. For example, Davis et al (2004) found that pollen limitation led to reduced population growth rates in low density populations of the invasive grass *Spartina alterniflora.* Distance to nearest neighbors was also found to be correlated with pollination success and seed set in the Canadian yew[10]. Given the sparse information currently available, researchers have called for more studies to investigate pollen limitation in a variety of wind-pollinated taxa[4]. My proposed project to determine pollen limitation in *D. leibergii* answers this call for further studies.

Through this project, I will address the following questions:

1) What is the seed set in a prairie remnant and how does it relate to density of flowering plants?
2) What is the extent of pollen limitation in the prairie remnant?
3) Does self or outcross pollen yield higher seed set? How does this affect pollen limitation?

I will determine natural seed set in two sites with differing densities of *D. leibergii* to assess and compare the reproductive success of the two populations. To assess pollen limitation, I will treat plants with supplementary pollen; if the seed set is higher in pollen supplemented plants than naturally pollinated plants, then the populations are pollen-limited. I will also conduct a pollen exclusion experiment, in which pollen-excluded plants only receive self pollen. This experiment will determine if plants are more compatible with self or outcross pollen.

I will conduct the experiments at two sites at Hegg Lake, a prairie remnant owned by the Minnesota Department of Natural Resources. I had obtained permission to use the site last summer, so I will obtain permission again this summer. Starting in June, I will lay a transect at each site, randomly select and tag 30 *D. leibergii* plants along each transect, and randomly assign each plant to one of three treatments: 1) open (natural) pollination, 2) pollen supplementation, 3) pollen exclusion (self-pollination). To estimate plant density, I will measure the distance of the nearest neighbor to each plant. For plants in the exclusion treatment, I will place a parchment bag over the tallest budding inflorescence on each plant (a single inflorescence has multiple buds; see Appendix 1), seal the bag around the stem, and attach the bag to a supporting stake. Every 2-3 days, I will visit all plants at both sites to check the maturity of the anthers. I will record the dates when anthers and stigmas emerge. When anthers emerge in florets assigned to pollen supplementation, I will attempt to remove the anthers using forceps, though it might not be practical given the small size of the anthers and potential damage to stigmas. When stigmas emerge in florets assigned to pollen supplementation, I will lightly brush the stigmas with pollen-donor anthers. The pollen-donor anthers will be harvested earlier on the same day from distant plants and collected in sterile centrifuge tubes. Although *D. leibergii* blooms throughout the summer, blooming rates gradually decline after mid-July, so I expect to complete the pollen supplementation experiment by early August. When the seeds are mature, I will harvest and store them in coin envelopes. I will bring them back to the Chicago Botanic Garden lab to determine seed set by weighing, dissection, or X-ray scanning in fall quarter. After obtaining seed set data, I will carry out data analysis to compare seed set among treatments using generalized linear models with binomial response.

My coursework and experience in the field and laboratory have provided me with a strong background in the concepts of prairie ecology and restorations as well as their practical applications. I have taken several classes with a focus on conservation and prairie ecology (BIO347 (Conservation Biology), BIO346 (Field Ecology), and EPC394 (The Policy and Science of Environmental Restorations)). I have also learned how to carry out statistical analyses in BIO 313 (Quantitative Methods for Ecology and Conservation) during winter quarter. This spring, I will be taking BIO330 (Plant Biology), BIO349 (Plant Community Ecology) and BIO323 (Conservation Genetics), which will further enrich my knowledge of plant biology and ecology. In regards to field experience, I learned various field methods during my internship in Minnesota last summer, including hand-pollination of *Echinacea angustifolia* (narrow-leaved purple coneflower) in a pollen compatibility experiment. I have been working in Dr. Wagenius' ecology lab at the Chicago Botanic Garden since fall quarter, so I am also familiar with seed weighing, scarification, and X-ray procedures to determine seed set. I am confident that I have all the necessary background and skills to carry out my proposed experiment and subsequent data analyses.

This project will allow me to pursue a deeper understanding of the ecology and reproductive biology of *D. leibergii*, the species which is the focus of my BIO 399 independent research project[11]. I am investigating the variation of quantitative seed and seedling fitness traits in *D. leibergii* among populations, using seeds that I had harvested from several prairie remnants (including Hegg Lake, my proposed site) during my internship. Hence, I am very familiar with the morphology of *D. leibergii* seeds and floral parts. During the fall and winter, I germinated some of these seeds in a pilot study to determine methods to maximize germination. Using a combination of treatments, I was able to obtain germination rates that were higher than those reported in previous germination trials[3]. However, I was concerned by the overall low germination rate[3]. This problem was the primary motivation for my proposed project. I am looking forward to learn more about this fascinating species through this project.

After completion of this project, I plan to write up my results and present them at the Undergraduate Research and Arts Exposition and Midwest Ecology and Evolution Conference (MEEC) next year. This project will also be the subject of my senior thesis. Since I plan to pursue a M.S. in Plant Biology and Conservation at Northwestern following graduation, this project will allow me to develop research skills for graduate research. Lastly, these research skills will stay with me for life and help me achieve my ultimate goal of becoming a conservation biologist.

Literature Cited and Notes

[1] Ashman, T. L., Knight, T. M., Steets, J. A., Amarasekare, P., Burd, M., Campbell, D. R., Dudash, M. R., et al. (2004). Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology*, *85*(9), 2408–2421. Eco Soc America. Retrieved from http://www.esajournals.org/doi/abs/10.1890/03-8024

[2] Milburn, S. A., & Bourdaghs, M. (2007). Floristic Quality Assessment for Minnesota Wetlands - Appendix A: Minnesota's Wetland Species. (Minnesota Pollution Control Agency, Ed.). St. Paul, MN. Retrieved from http://www.pca.state.mn.us/index.php/view-document.html?gid=13347

[3a] The Millenium Seed Bank at Royal Botanic Garden, Kew conducted preliminary trials with *D. leibergii* and obtained germination rates of 22.8-23.8%, but results were not statistically significant due to small sample size (email correspondence, Vanesssa Sutcliffe, Training Specialist).

[b] Cathy Thomas, a propagator at Chicago Botanic Garden (personal correspondence) obtained very low germination rates (her estimate was 5%) for *D. leibergii* with standard germination procedures.

[c] From my germination trials with bulk *D. leibergii* seeds, I obtained mean germination rates of 4.4% (untreated seeds), 20.6% (stratified), and 29.5% (scarified). Overall germination rate was 17%.

[4] Friedman, J., & Barrett, S. C. H. (2009). Wind of change: new insights on the ecology and evolution of pollination and mating in wind-pollinated plants. *Annals of botany*, *103*(9), 1515-27. doi:10.1093/aob/mcp035

[5] Knapp, E. E., Goedde, M. a., & Rice, K. J. (2001). Pollen-limited reproduction in blue oak: implications for wind pollination in fragmented populations. *Oecologia*, *128*(1), 48-55. doi:10.1007/s004420000623

[6] Koenig, W. D., & Ashley, M. V. (2003). Is pollen limited ? The answer is blowin ' in the wind. *Evolution*, *18*(4), 157-159.

[7] Bertness, M. D., & Shumway, W. (2012). Consumer driven pollen limitation of seed production in marsh grasses. *America*, *79*(3), 288-293.

[8] Davis, H. G., Taylor, C. M., Lambrinos, J. G., & Strong, D. R. (2004). Pollen limitation causes an Allee effect in a wind-pollinated invasive grass (Spartina alterniflora). *Proceedings of the National Academy of Sciences of the United States of America*, *101*(38), 13804-7. doi:10.1073/pnas.0405230101

[9] Gleaves, J. T. (1973). Gene flow mediated by wind-borne pollen. *Heredity*, *31*(3), 355-366. The Genetical Society of Great Britain. Retrieved from http://dx.doi.org/10.1038/hdy.1973.91

[10] Allison, T. D. (1990). Pollen production and plant density affect pollination and seed production in Taxus canadensis. *Ecology*, *71*(2), 516–522. JSTOR. Retrieved from http://www.jstor.org/stable/1940305

[11] This independent study is funded by the Academic Year Undergraduate Research Grant from Winter 2011 to Spring 2012.

Appendix 1: Photos of *D. leibergii* inflorescence and floret structures.

A. A flowering inflorescence of *D. leibergii*. (Photo credits: Maria Wang)

B. Close-up of a single floret on a *D. leibergii* inflorescence. The violet brush-like structures are the stigmas, while the smaller, dark-colored structures hanging away from the stigmas are the anthers. (Photo credits: Joshua M. Drizin)

Appendix 2: Proposed Budget

Expenses	Costs
Travel: To and from Chicago Botanic Garden $1.75 per bus trip - 30 round trips (Fall Quarter)	$100.50
Pollination bags	$50.00
Microfuge tubes	$15.00
Forceps	$11.00
Microbrushes	$11.00
Coin envelopes	$10.00
TOTAL	**$197.50**