

Report to the San Diego Orchid Society 2013

A study of the conservation genetics and future viability of a rare North American orchid, (*Platanthera praeclara*).”

Dr. Steve Travers, North Dakota State University

Funding:

I received funds from the San Diego Orchid Society to support my research on the Western Prairie Fringed Orchid during the 2012-2013 field seasons. These funds were used to support graduate students conducting field work on the orchid and supplies for genetic analysis of wild populations. The support of the San Diego Orchid Society has been crucial for the development of the project that has led to two publications in peer-reviewed journals and the development of a long term research program that is providing much needed insights into the conservation of this threatened species.

Overview

The leading cause of decreased biodiversity in response the actions of humans is generally considered to be habitat loss and habitat fragmentation (Figure 1). By converting wild environmental spaces to areas beneficial to humans the landscape around the world has been altered dramatically everywhere humans have migrated and there is ample evidence of major impacts on biological organisms including reductions in biodiversity from increased extinction rates.

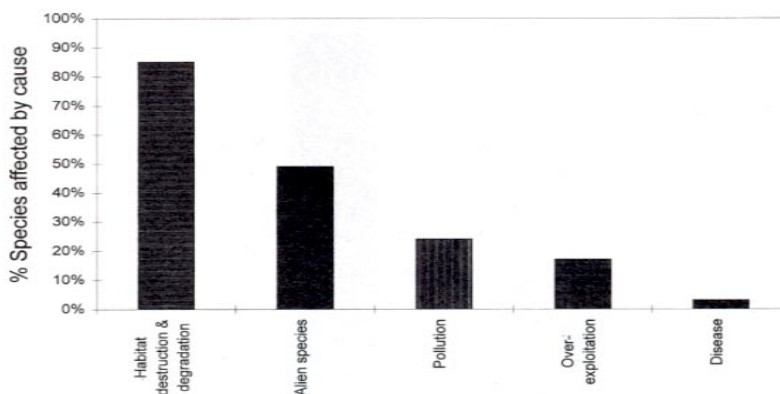


Figure 1. Causes of species rarity

What does habitat loss do to a biological species? Habitat loss has two effects that ultimately can impact the chance a species will go locally or globally extinct. Both theory and empirical studies have shown that habitat loss can lead to smaller population sizes and increased fragmentation of the available habitat. In turn these two effects negatively impact populations of organisms by increasing the role of genetic drift in fixing random alleles in populations and isolating populations from gene flow with other populations. Inbreeding is expected to increase with isolation and smaller population sizes because closely related individuals become a larger proportion of individuals available for mating. The culmination of these cascading effects is a reduction in the viability of populations because of negative genetic consequences. Inbreeding decreases the fitness of individuals and reduced population genetic diversity due to genetic drift can both decrease the long term viability of populations. Michael Soule referred to this series of negative impacts on the population genetics of species as an extinction vortex because once populations are reduced and isolated they can spiral into extinction. Species with habitats that have been heavily impacted by humans are at increased risk for these cascading effects.

Tallgrass prairie

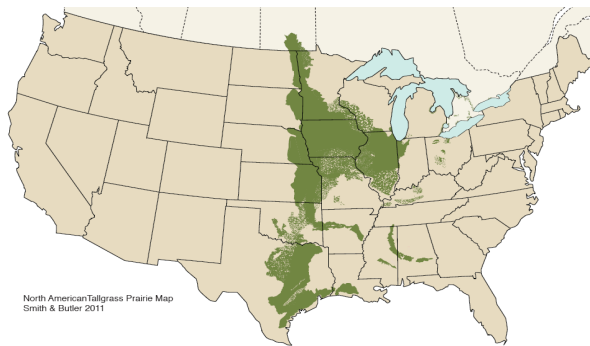


Figure 2. Original distribution of tallgrass prairie

Of all the ecosystems in North America that have been heavily impacted by habitat loss, none stands out as much as tallgrass prairie (Figure 2). The tallgrass ecosystem has been almost completely converted to cropland leaving the species adapted to living in this ecosystem in a highly fragmented landscape. Tallgrass prairie is a subset of the grasslands of the great plains of North America stretching down from Canada along the eastern most edge of the plains down into Texas. The characteristic grass species like big bluestem that give it its name are exceptionally productive because of the relatively high amounts of annual precipitation relative to farther west into the rain shadow of the Rockies. This moisture level and the soil type found in tallgrass make it ideal for crops. As a result an estimated 97% of it has been converted to agricultural fields. What remains are remnant patches that are generally in areas too rocky to plow or in

preserves. Many of the plant species that grow in tallgrass prairie are found elsewhere limiting the impact of habitat loss.

The Western Prairie Fringed Orchid

Probably the most charismatic of the species that grow in these regions is the western prairie fringed orchid – *Platanthera praeclara* (Figure 3).



Figure 3. *Platanthera praeclara*

This species, called a charismatic mega-flora, is an ideal species to examine the effects of habitat loss on the genetic characteristics of populations because it historically has been found in close association with tallgrass prairie. It is the westernmost species out of a handful of species in the genus stretching across the eastern half of the continent. *P. praeclara* and *p. leucophylla* are a species pair that differ from the other platantheras to the east in that they are white, moth pollinated and generally found in grasslands versus the forested habitats of the other species. These two species are also far more rare than the eastern species. Both of the fringed orchids are listed as threatened at the federal level because their numbers have dropped off so precipitously.

P. praeclara is dependent on extremely wet environments and is really only found in wetland swales where the ground is uneven and water levels can vary from year to year. Collection data from herbaria indicates that historically it was found in counties from Oklahoma to Canada.

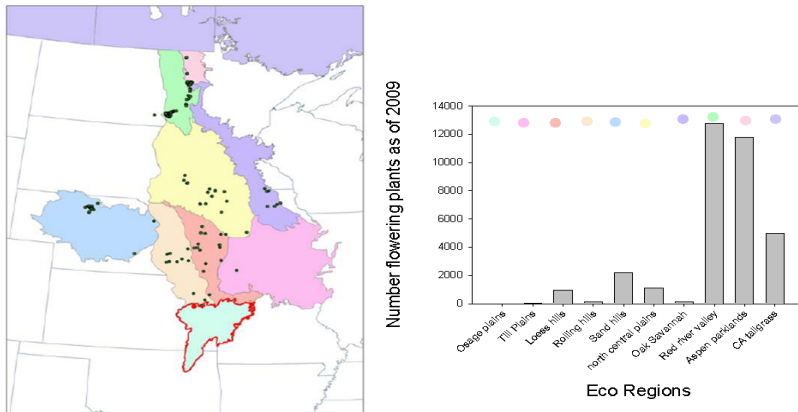


Figure 4. Current distribution of *P. praeclara*

Current censuses summarized by the USFWS indicate it is now found in isolated remnants of tallgrass prairie wetland in populations ranging from one flowering individual to thousands (Figure 4). As such, it has two characteristics that potentially make it susceptible to population viability problems. Two key parameters determining long term viability of small populations emerging from theoretical work by Lande and Pulliam are the proportion of land around a species that is suitable as habitat and the dispersal ability of that species. Greater dispersal ability of a species can allow it to overcome habitat loss and fragmentation all else being equal.

P. praeclara potentially has two strikes against it because it is an obligate wetland species adapted to tallgrass prairie it has lost a lot of the suitable habitat originally available to it. This specialization on wet areas in a highly converted landscape also means that individual populations can be relatively long distances from one another. Dispersal of seeds and pollen among populations is poorly understood in this species. So to better forecast the long term viability of the species in the face of habitat fragmentation we need to answer these questions: are the small populations and the isolation of those populations reducing the long term viability of populations by decreasing the genetic quality of those populations? Here I am referring to genetic diversity and inbreeding within populations. This work was done in collaboration with graduate students Andrew Ross working on his Masters and Kirk Anderson and Marion Harris from entomology. We began the study by developing microsatellite molecular markers specifically for the WPFO with the help of a lab at the University of Georgia. From leaf samples collected in ND and MN they isolated 31 microsatellite markers. We further screened those and found six loci that were both variable and not in linkage disequilibrium. We then used primers for those six loci to characterize a series of populations in the the area (Figure 5) in 2009 and 2010.

Study Populations

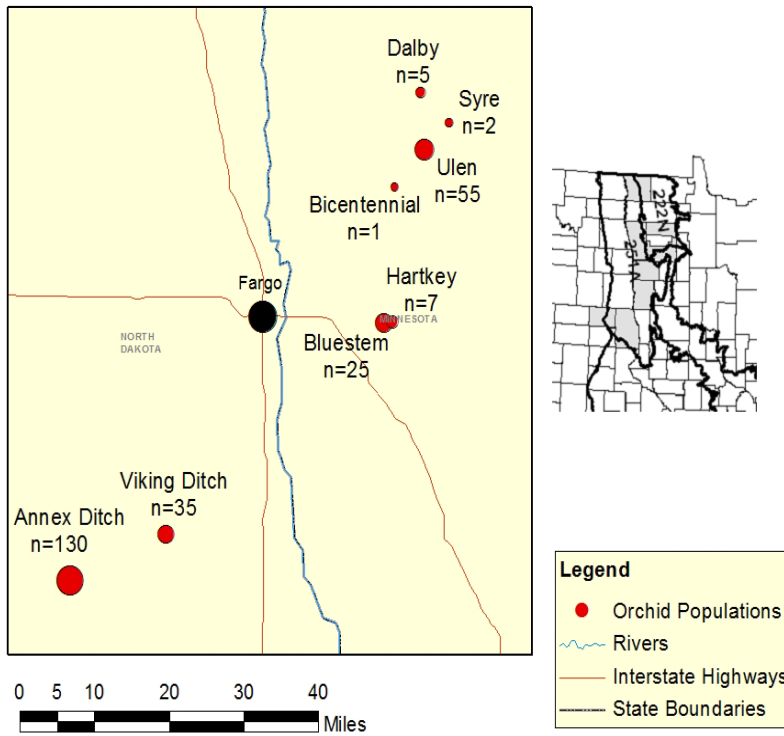


Figure 5. Study sites

As a result of the microsatellite analyses of these populations we concluded that there was no real evidence for low genetic diversity in these populations (Figure 6).

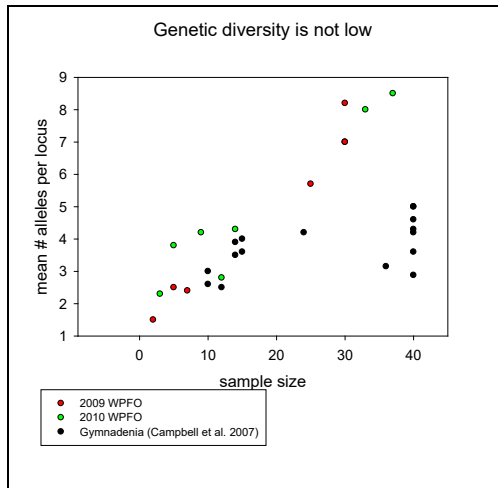


Figure 6. Genetic diversity levels by sample size

Our next question though had to do with dispersal . Could we find any evidence that these populations are in fact isolated from one another and beginning to diverge which would be a sure sign that pollen and seeds were not moving between populations. As I mentioned not a lot is known about propagule dispersal in this species. The seeds are absolutely tiny and capable of being born on air currents however it has also been suggested that water may be the most likely way seeds are transported. That would of course prevent movement between wetlands.

The pollen however is a different story. *P. praeclara* is pollinated by moths and exclusively by moths or more specifically hawk moths (Figure 7).



Figure 7. Hawkmoth pollinators of *P. praeclara*

Based on the work of Marion Harris and her lab it is known that six Species of sphingidae visit *P. praeclara* and are necessary for successfully pollen transfer. The reason is two fold: first the length of the nectar spur prevents small tongued insects from visiting and getting a reward and second, the pollinia which house the pollen must be transferred by a relatively large insect because of their placement. These plants are highly specialized mutualists with hawk moths. Hawk moths are strong flyers and can travel long distances in a night. However, they do visit

other species than *P. praeclara* and it is not clear if they can locate small populations of a couple plants.

A secondary concern is that rather than moving pollinia among widely separated populations, moth pollinators may very well be contributing to inbreeding in this species by self pollinating plants with their own pollen or with nearby relatives. *P. praeclara* is self compatible and a given plant will have up to ten flowers open at a time on an inflorescence. If a moth moves from flower to flower within the plant the possibility of geitonogamous self pollination is high.

So we wanted to know if there was a) a genetic signal for divergence among populations meaning limited dispersal between populations and b) evidence of inbreeding in these populations.

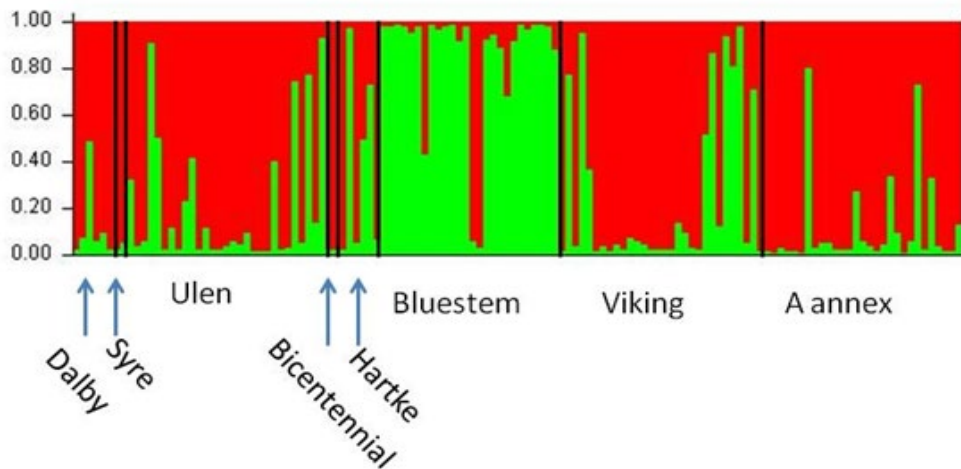


Figure 8. Structure analysis of *P. praeclara* populations indicating little divergence among them

By using standard population genetic analyses and statistics we found that F_{st} values (Figure 8) were relatively low which along with AMOVA indicated there was little divergence among pops. Despite the distances among the populations the microsats seem to suggest there was no evidence of serious isolation among the populations which may well be due to plenty of dispersal of pollen. Structure analysis indicated only one population was fairly distinct and this was in the middle of the range. The results in the second year were fairly similar.

The populations are not exceedingly different from each other genetically. However, when we assessed the observed and expected levels of heterozygosity we found that there seems to be an excess of homozygotes in all populations. Increased homozygosity is a typical consequence of inbreeding and indeed the inbreeding coefficients were significant suggesting that moths are in fact self pollinating plants or that crossing is between closely related plants. The overlap in alleles and allele frequencies between all populations with the exception of Hartke indicates a lack of isolation among populations but there are two possible explanations for this beyond

movement of pollen among populations. One is that seeds move between populations but pollen doesn't. This doesn't seem likely since Hartke is in the middle of the range. Second the allele frequencies may in fact reflect a historical pattern of founding. If all populations except Hartke were originally part of the same population and there has been insufficient time for the subpopulations to diverge we would see these patterns. In any case we found evidence that selfing and inbreeding could be a common phenomenon in this species.

Our next question was, If self pollination is common is that a problem? Some plant species produce seed through selfing and experience no decrease in fitness of individuals. Others suffer inbreeding depression as a result of increased homozygosity of deleterious alleles. So we conducted a field experiment to compare the results of selfing versus outcrossing.

We designed an experiment where we hand pollinated flowers on plants in the field and monitored their seed and fruit characteristics over time (Figure 9).

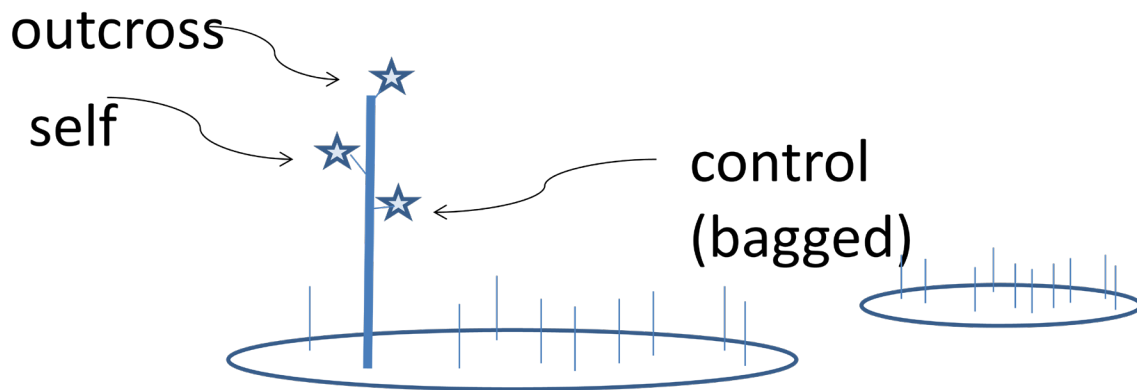


Figure 9. Experimental design

The plants we manipulated in this experiment were naturally occurring at a Nature conservancy site in North Dakota where there was a northern population of less than a hundred plants separated from a southern population by about 1 km. We marked 15 plants haphazardly in each population with metal markers and established three pollination treatments on each plant. One flower from each plant was hand pollinated with pollinia collected from the other population – outcross treatment; one flower was hand pollinated with pollinia from the same flower and a third flower was left in a pollinator enclosure bag the duration of the experiment for a control.

We found that selfing did not necessarily have an observable consequence on the seed pods – they still inflated. But we found the seeds were generally smaller and much less likely to have embryos (Figure 10).

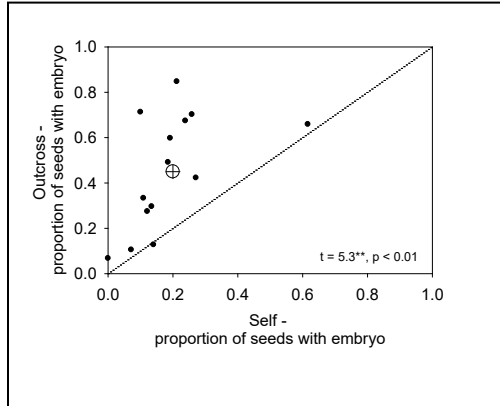


Figure 10. There are higher proportions of seeds with embryos in outcross versus selfed fruits

So it looks like inbreeding depression is very likely in this species. We also had some anecdotal evidence that selfing happens commonly in the WPFO. The fruit and seed characteristics are often similar to those from our self pollinations.

So the impacts of habitat loss and fragmentation may well include increased inbreeding and ultimately negative fitness aspects associated with inbreeding depression, especially in smaller populations (conclusions).

My Two goals for the future are to expand the range of our sampling to include Canada and farther south to see if we will eventually detect structure among the populations and ecological factors that may be isolating the populations. Secondly , I would like to conduct a comparative study with four other *Platanthera* in the same clade and address to what extent rarity has been the result of pollinator syndrome and habitat.

Products from this research:

Ross, A.A., L. Aldrich-Wolfe, S. Lance, T. Glenn, S.E. Travers. 2013. Microsatellite Markers in the Western Prairie Fringed Orchid, *Platanthera praeclara* (Orchidaceae). *Applications in Plant Sciences* 1:1200413.

Fox, K., P. Vitt, K. Anderson, G. Fauske, S. Travers and M.O. Harris. 2013. Pollination of a threatened orchid by an introduced hawk moth species in the tallgrass prairie of North America. *Biological Conservation* 167:316-324

Andrew Ross, Masters in Science, North Dakota State University
