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Additional case-studies from around the globe

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Propagation and re-introduction of the western prairie fringed orchid in Nebraska, USA

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Introduction

The western prairie fringed orchid (*Platanthera praeclara*) is an endemic orchid that is protected throughout its range in the Great Plains of the United States and Manitoba Province in Canada. The orchid is on the IUCN Red List for North America. The species had not been successfully propagated prior to 1999, when it was successfully micropropagated from seeds at the plant research lab at Omaha's Henry Doorly Zoo and subsequently re-introduced to augment an existing wild population. The research project was initiated in the sandhills of Nebraska where the largest known population in the state is located on the Valentine National Wildlife Refuge. The orchid species is sometimes found in widely scattered and isolated populations throughout the eastern two-thirds of the state but most of those populations have only a few individual plants. The species is associated with a specific pollinator which is believed to be in decline across the range and the orchid is also highly dependent on a symbiosis with specific soil fungi which facilitate uptake of soil nutrients for plant survival.

Western prairie fringed orchid (*Platanthera praeclara*)

The research project involved propagation and re-introduction of the propagated orchids, isolation of the fungi associated with the orchid's roots and rhizomes in the wild and soil nutrient analyses of the natural habitat in order to identify environmental conditions necessary for survival. The primary aim was to create a profile of some of the factors in the environment that support the orchid and to assist wildlife managers in decision-making regarding currently known populations and identifying potential re-introduction sites.

Goals

- Goal 1: Propagate the orchid *in vitro* for use in wild population augmentation.

- Goal 2: Develop successful micropropagation protocols for the orchid seeds.
- Goal 3: Isolate the symbiotic fungus(i) from underground tissues of the orchids found in the wild.
- Goal 4: Analyze soil nutrients present in the orchid's natural habitat.
- Goal 5: Re-introduce micropropagated juvenile orchids to the wild.

Succes Indicators

- Indicator 1: Successful propagation of the species that had previously resisted attempts at seed propagation.
- Indicator 2: Isolation and characterization of suspected fungal symbionts.
- Indicator 3:Determining nutrients and minerals in soils at orchid sites and comparing them with nearby non-orchid sites.
- Indicator 4: Monitor re-introduced plants for growth and survival.

Project Summary

Due to its protected status under the US Endangered Species Act permits were obtained from US Fish and Wildlife Service and the Nebraska Game & Parks Commission to collect seeds from *Platanthera praeclara* on the Valentine National Wildlife Refuge. The terrestrial species makes a very sporadic appearance from one year to the next and is believed to survive underground for some of its life stages, which may be part of a species survival strategy in a harsh environment characterized by broad swings in temperature and rainfall. The orchid's sporadic show may also be related to a periodic unavailability of the suspected fungal symbiont(s) during natural fluctuations of surface waters that peak and recede within the habitat over the course of the seasons and years. The habitat is characterized by arid hills with low-lying sub-irrigated meadows between the sparsely grass- covered sandhills.

The orchid seeds are smaller than a single grain of dust and have both physiological and chemical dormancies which must be understood in order to get them to germinate. A multi-step process was developed to scarify and surface sterilize the seedcoats without damaging the bare microscopic embryo within prior to *in vitro* culture on sterile agar-gelled media. The germination is very low and generally was less than 6% and the sensitive seedlings were prone to easy die-back even under sterile *in vitro* conditions and were slow-growing. Juvenile plants used for re-introduction trials were grown *in vitro* at the lab for two to three years prior to planting-out. The orchids were kept in vitro under sterile conditions to reduce any chance of introducing pathogens to their specialized microhabitats in the wild. More than one hundred-thirty juvenile orchids were planted back in the habitat near the adult plants which provided the seeds that were collected three years earlier. Re-introduced orchids survived at a low rate but were encouraging enough to warrant further re-introduction investigation for the species.

To identify potential symbionts, a small amount of root tissue was collected in the wild and the fungi were then isolated in the laboratory. A total of twenty-seven isolates were cultured *in vitro* and fourteen of them were targeted as possible symbionts for the orchid. A small number of the *in vitro* grown orchids were inoculated with the suspected symbionts. Inoculated orchids grew equally well as

Orchid habitat in the Nebraska sandhills

non-inoculated orchids for a few weeks but those inoculated were more likely to die before maturity than the orchids that were grown in the absence of fungal inoculation. Soils were analyzed from orchid sites and nearby non-orchid sites to determine whether there were nutrients more or less prevalent in the orchid microhabitats. The soil analyses may help make it possible to test potential re-introduction sites for their soil contents prior to planting out *ex situ* produced orchids. Soil core samples were taken near adult *P. praeclara* orchids and at non-orchid sites nearby which appeared to be similar to the orchid sites. Soil cores were taken in spring, summer and in the fall and soil samples were almost always totally water-saturated when taken near an existing orchid, regardless of the season, while samples taken in similar-looking non-orchid habitat within 50 m of orchids were not saturated, indicating that water availability is critical to the orchids' survival. As a result of the soil analyses a general profile of nutrient, soil textures and water availability have been delineated for future *P. praeclara* re-introductions if, and when, they are made in this part of the orchid's native range.

Major difficulties faced

- A complicated and prolonged legal permitting process and many restrictions placed on the project since the study took place on federal land.
- Determining the best time to re-introduce the orchids to the natural environment when repeated measures were restricted by governmental regulations.
- The orchid species is extremely sensitive to root disturbance making handling of the seedlings difficult.
- Identification of preferred re-introduction sites.
- A concurrent eight-year drought was assumed to have had a detrimental effect on the re-introduced plants and may have skewed the outcome to some degree.

Major lessons learned

- The orchid species can be successfully propagated in asymbiotic cultures.
- Re-introductions of inoculated orchids were no more likely to survive after re-introductions than orchids that were raised asymbiotically *in vitro* and re-introduced to the wild.
- The species shows a preference for soils that are generally nutrient-poor but the element magnesium is abundant near existing *P. praeclara* orchids.
- Re-introductions are best made in the very early spring when the soil and air temperatures are still cool and there is ample soil moisture.

Success of project

Highly Successful	Successful	**Partially Successful**	Failure
		√	

Reason(s) for success/failure:

- Survival of re-introduced orchids was generally somewhat lower than expected.
- Legal restrictions precluded adequate replications for the re-introductions which would have allowed a large study to be done.
- More than 98% of soil microbes are still unclassified by science, making fungal symbiont identification difficult.