DROUGHT, PERIL, AND SURVIVAL IN THE GREAT PLAINS: CYPRIPEDIUM CANDIDUM

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The small white lady's slipper, *Cypripedium candidum*, is a rare sight in Nebraska. The shy, diminutive orchid is found in only seven locations scattered widely across the state, and although some populations may have a hundred or more blooming individuals, species protection is critical for its long-term survival. The specific name *candidum* comes from the Latin word *candere*, which means "to shine or to glow". In the sixteenth century lady slipper orchids were called "Slipper of Our Lady", which was meant to pay homage to the mother of Christ (Simon, 1975). The conversion of tall-grass prairies to productive cropland over the last one hundred years left little more than a few native prairie remnants intact, so that now the encounter of even one orchid in bloom is a gem not soon forgotten.

Populations in the state represent some of the most western locations within the range of *Cypripedium candidum*, making the genotype(s) important conservation subjects. Orchids face a precarious existence in the harsh climate of the Great Plains, where frequent droughts, bitterly cold, dry, winter winds and blazing summer temperatures test a species' mettle. Drought hit the plains particularly hard in recent years, and much of Nebraska has now experienced as much as 8 years of precipitation shortfall. That the orchids survive at all, under such stress, is simply amazing. Herbivores like to browse the orchids, often removing the tops before they can produce mature seeds to ensure a succeeding generation. Human safety takes precedence in road maintenance, sometimes permanently altering the orchids' habitat, as happened for *C. candidum* in Nebraska in 2005. When orchids are

displaced by human activities, rescue operations are often the only option left; even if not the ideal. The human aspects are a fact of modern life, and will continue to put pressure on natural habitats. When an orchid population is faced with destruction what can orchid enthusiasts do? Some options are; to notify the appropriate governmental agencies, discuss with landowners how to protect the habitat, inquire about a conservation easement for the property, individuals or organizations can inquire about purchase of the site, one can move the orchids out of harm's way if permission is granted, or propagate and preserve some germplasm representative of the population so that the genetic diversity assumed present in the population does not disappear forever. Ideally, genetic studies should also be completed before any long-ranging decisions are made, but the reality is that destruction of habitat often happens with little or no warning, leaving no time to conduct all the research in advance.

Two of the seven known *Cypripedium candidum* populations in Nebraska now face an uncertain future due to land sales and development, and a third faces environment-altering practices that may destroy the population. One of those populations is on the edge of a medium sized city where the orchid's habitat is scheduled for commercial development. All the sites have relatively good soil, which can easily be converted to crop production.

The species as found in Nebraska, displays a range of variations, with some individuals possessing yellow sepals and petals with maroon-brown venations (Fig. 1), some having green sepals with maroon dots and venations, and some whose petals and sepals are entirely maroon-brown.

Others have reported the maroon colorations as possible evidence of the relationship of *Cypripedium candidum* to *Cypripedium parviflorum* (Smith, 1993). An overlap of ranges for *C. candidum* and *C. parviflorum* is believed to result in a natural hybrid known as *Cypripedium Xandrewsii* Fuller. In Nebraska the nearest population of *C. parviflorum* is found within a woodland setting more than one hundred twenty five miles away from any of the sun loving *C. candidum* populations, although at some point in history the two ranges for the species may well have overlapped. Nebraska's *C. parviflorum* population was just discovered in

the early 1990s, approximately 75 years after it was assumed extirpated from Nebraska, and botanists' early accounts of the state do not identify any overlap of the two species.

The labellum is very similar in each of the variations. It is creamy white with light markings of magenta pink in lines along the sides and bright splotches of magenta on the inner rim, as well as on the lower portion of the column, near the edges. The leaves are reported as clasping the stem, according to Doherty (1997), but as the growing season progresses in the Great Plains, the leaves become fully expanded creating a lovely, open form (Figures 3, 5 and 6).

Figure 1. C. candidum with mostly yellow-green petals and sepals.



Figure 2. Maroon petals and sepals on *C. candidum*





Figure 3. C. candidum plants in protected habitat at the Omaha's Henry Doorly Zoo



In bud, the labellum is most prominent (Figure 4), and does not yet show the magenta markings that will be visible on the opened flowers later in the season.

Figure 4. A rescued plant in bud on May 6th, 2006.

As the blooming season comes to a close the flowers senesce and begin to take on a translucent appearance (Figures 5 and 6), but the leaves persist and appear to continue photosynthesizing for 3-4 months longer, producing energy that is translocated to the underground root

structures where the flower and leaf meristems develop for the next growth cycle.



Figure 5. *Cypripedium candidum* as the flower senesces.

Rescued plants:

Road work resulted in the displacement and damage to a number of the plants by the roadside at one of the native habitats. With permission of the state, some of the rescued plants were placed in a protected outdoor location and several others were potted up and placed under greenhouse conditions in order to study the relative merit of rescue operations if, over time, more plants face eminent threat of destruction. Rescued plants often die quickly after removal from their

habitat (K. Kennedy, personal communication). The orchids were scraped from the roadside by road grader activity that damaged roots on most of them, and they were found covered with masses of debris at the bottom of the ditch (Figure 7). All rescued plants survived and 85% bloomed the first year, both the individuals planted outdoors as well as those that were potted up and grown under greenhouse conditions. It was decided to retain some of the native soil around the roots for those that were not bare-rooted when found and those specimens produced somewhat more vigorous growth over the growing season, as compared to those in the greenhouse.



Figure 6. A rescued plant as the flower begins to fade.

There are risks whenever orchids are removed from their natural environment, not least of which is that it is difficult to compensate for local conditions and adaptations, especially if plants are moved any great

distance. Those local adaptations are another important reason that germplasm in the form of plants, seeds or pollen, should be preserved by long-term means if possible, so that if the need arises and good habitat is available, preferably in the original area, plants representing the local wild type could then be used for reintroduction.



Figure 7. C. candidum plant emerging from the roadside debris.

Seed-banking the local wild-type:

Seeds are possibly the most efficient forms of germplasm preservation because they carry the possibility of genetic diversity and they are so tiny that great numbers of seeds can be stored within a very small space. Cryopreservation protocols are being worked out so that the diversity of Nebraska *Cypripedium candidum* populations will be represented and available for future conservation action plans. Until the time when efforts to keep wild populations safe and healthy are in practice, and the species' propagation and reintroduction methods are fully outlined, cryopreserved seeds provide materials for future research. Orchid seed cryopreservation protocols have to be researched on a species by species basis because each species has its own unique physical properties and culture requirements. The ability to preserve seeds for

years, or even decades, is a powerful tool for conservation, provided that along with implementation of those protocols we also learn to better protect remnant habitats, understand the pollinators, and continue to study the microbial associations that are accepted as critical for the species. Seeds for *C. candidum* have already been tested in cryopreservation and trials are being conducted on their germinability in post-thaw cultures in the laboratory. The project is preserving seeds collected from the population facing the most immediate threat of destruction in the state. Some of the seed testae are damaged in cryopreservation tests (Figures 8 and 9), but other embryos are able to withstand the extreme temperatures of liquid nitrogen (-196°C), and still germinate. Results from the project will determine whether this is a feasible means to preserve *C. candidum* seeds for future conservation actions in the state.

Figure 8. (left) Cryopreserved Platanthera sp. seeds, SEM at 194x.

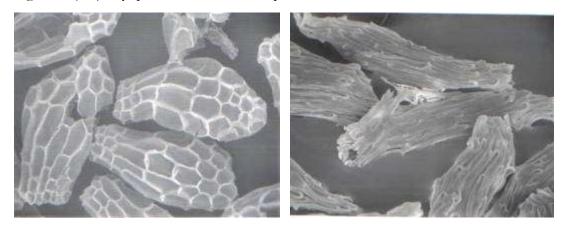


Figure 9. (right) Scanning electron micrographs (SEM) at 372x, of cryopreserved tropical orchid seeds.

If practical it would be ideal if each state, province or region could preserve local genotypes for the long-range future, whether in cryopreservation or in parent populations grown under protected conditions. Keeping the populations numbers large is important, since plant species that maintain large populations stand a better chance of attracting pollinators and surviving over the long term. In order to

maintain healthy populations there need to be increased incentives for habitat protection by private landowners, and research should be encouraged about pollination biology, edaphic changes, pollinator populations, mycorrhizal associations, soil conditions, and plant communities across the orchid's entire range in order to save the species. Above all, those who have knowledge about *Cypripedium candidum* are encouraged to educate others in the region about the species itself, and about what can be done to save these beautiful members of our natural legacy.

Acknowledgements:

My thanks are extended to Jim Pyrzynski and Tim Janssen of the Greater Omaha Orchid Society for their assistance and perseverance to save the Nebraska *Cypripedium candidum* wild-type. Gratitude is also extended to Mike Fritz, biologist for the Nebraska Natural Heritage Species program at the Nebraska Game & Parks Commission, and to Dr. Kathryn Kennedy at the Center for Plant Conservation in St. Louis, for their help and valuable information. Dr. Simmons, director of Omaha's Henry Doorly Zoo, continues to be an inspiration to all of us who know him, and the motivating force behind conservation efforts for threatened species.

Literature cited:

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Received on 4/23/07: As a postscript to the article about Cypripedium candidum rescued and planted at the zoo; I am happy to tell you that every one of them has reappeared above ground this spring. And most of them are displaying more ramets than last year. We are, of course, hoping that they will persist over the coming years.

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Glossary:

cryopreservation: preservation of seeds or tissues at extremely low temperatures which in effect is supposed to stop metabolism, so that cells don't age

edaphic: plant communities influenced by the soils, not just the climate (i.e.

effects from sandy soils, acidic soils, or nutrient-poor soils)

germplasm: an organism's DNA (i.e. seeds are stored DNA in a frozen seedbank) *meristems*: clusters of cells at zones that will eventually produce shoots or roots *testae*: seedcoat