POPULATION AND HABITAT VIABILITY ASSESSMENT FOR THE WESTERN PRAIRIE FRINGED ORCHID
(*Platanthera praeclara*)

Eugene Mahoney State Park
Ashland, Nebraska
27-30 April 1997

Final Report
13 October 1997

Sponsored By:
Omaha's Henry Doorly Zoo
Nebraska Game and Parks Commission

In Collaboration With:
The Conservation Breeding Specialist Group (SSC/IUCN)

A contribution of the IUCN/SSC Conservation Breeding Specialist Group in collaboration with Omaha's Henry Doorly Zoo and Nebraska Game and Parks Commission.

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POPULATION AND HABITAT VIABILITY ASSESSMENT (PHVA) FOR THE WESTERN PRAIRIE FRINGED ORCHID (*Platanthera praeclara*)

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Section 1
Executive Summary

EXECUTIVE SUMMARY

The western prairie fringed orchid (*Platanthera praeclara*) was listed as a threatened species under the federal Endangered Species Act in 1989 as a result of a decline in the species' range over the past 100 years, the reduced size of the remaining populations, and an increasingly fragmented distribution. Approximately 74 sites are known in 38 counties over 7 states. Historically, the species was more widely distributed and was locally common. The species is endemic to the North American tallgrass prairie community and the Nebraska Sandhills but has been found occurring naturally in disturbed sites associated with this habitat. Major threats to the species' persistence are the loss of habitat through conversion to agricultural uses, population fragmentation, incompatible land use practices, hydrologic changes induced by human activity and invasion of suitable habitat by exotic plant species. A revised federal recovery plan for the species was distributed in early spring, 1997.

The orchid was recognized to be in decline as early as 1898 in the state of Nebraska and was listed as a state threatened species in 1989. The species has continued to decline and based on cumulative data in 1997, currently consists of 271 ramets distributed in 14 known populations in Nebraska. These populations are distributed in 8 Nebraska counties: Cherry County with 3 sites, one of which is regarded as a metapopulation; 2 sites in Lancaster, 3 in Otoe, 2 in Pierce, and one site each in Saline, Sarpy, Seward and Hall counties. These sites are in habitats of 3 different ecotypes and may possibly represent genetically distinct, locally adapted populations. A common feature of many of the sites is sub-irrigation by near-surface groundwater. The extent of population fragmentation and the disjunct distribution suggest that most populations are probably too small for evolutionary viability. The species will need active management and protection to survive and increase at all of the sites. In addition, the requirements for seed germination and seedling development are currently unknown. This lack of information limits propagation of the species for conservation, research or commercial purposes.

Initial discussions concerning the application of the Population and Habitat Viability Assessment (PHVA) workshop process to the western prairie fringed orchid in Nebraska occurred between the Nebraska Game and Parks Commission, Omaha's Henry Doorly Zoo and the Conservation Breeding Specialist Group (CBSG) in September 1996. The Nebraska Game and Parks Commission formally invited the Conservation Breeding Specialist Group on 22 October 1996 to conduct a PHVA workshop for the species in Nebraska. The Henry Doorly Zoo agreed at that time to provide personnel, organizational, financial and logistical support for the workshop. The PHVA was held at Eugene T. Mahoney State Park in Ashland, Nebraska, 27 - 30 April 1997. Forty- five participants were present, representing nearly the entire range of the species including Iowa, Kansas, Minnesota, Missouri, Nebraska, North Dakota and Manitoba, Canada. Most governmental and non-governmental agencies involved in various aspects of conservation, research or management of the orchid over its range were represented. Stakeholders from within Nebraska included private land owners, non-governmental organizations, universities, state and federal agencies working in the state and multiple representatives from within Nebraska Game and Parks Commission. People with diverse expertise were present at the PHVA, including habitat managers, field researchers, biologists, botanists, geologists, hydrologists, and geneticists. Mike Fritz of Nebraska Game and Parks Commission and Lee Simmons, Director of Omaha's Henry Doorly Zoo, opened the meeting. Ulysses Seal, Chairman of CBSG, gave an overview

presentation covering species extinction, the history of conservation, and the philosophy of CBSG. He then addressed specific issues concerning small population biology and dynamics and discussed organizational structure and function as it relates to the conservation of endangered species. Following this opening session, a series of papers were presented on current topics in orchid conservation by a number of orchid experts. Text of these papers can be found in Appendix I of this report.

Working groups were formed to address the primary areas of concern for the participants: distribution and field survey of Nebraska populations, life history, *ex-situ* propagation, land use and habitat management, and population dynamics and modeling. The working groups accumulated known information regarding the species and issues of concern surrounding it, evaluated the information and identified areas of insufficient information or areas of concern and formulated research recommendations or management actions to resolve those issues. Over a three-day period the working groups met in continuous session throughout each day, with one- to two-hour plenary sessions daily in which each group summarized their results and discussed the issues raised surrounding their topic. Each working group produced a detailed written report and recommendations that were individually reviewed in plenary session and discussed in detail. Consensus was reached on each recommendation to be included in the workshop report.

Several issues became clear in the course of the discussions. For example, virtually no specific life history information is available for the species. The life history working group ascertained that although some of the orchid populations have been studied extensively, we still know very little about some crucial biological details of this species in any population. The modeling working group demonstrated that these small populations could be highly vulnerable to extinction and that life history variables, such as seed set and rate of seed germination, can profoundly influence growth dynamics. These factors are also crucial in determining the management methods that will enable the populations to survive. In addition, it was apparent that there are significant differences between populations located in various climatic and geologic conditions and, based on the modeling group's work, these differences can profoundly effect the population's survivability. The absence of distribution information was considered to be an important issue effecting the preservation of the species in Nebraska. The *ex-situ* working group addressed a wide range of issues including *in-vitro* propagation of the species, education programs and collaborative research efforts. This group also addressed other issues concerning management of the species such as translocation and reintroduction into habitats where the orchid occurred historically but is not found at the present time. Translocation and reintroduction were recognized as complex programs requiring consideration and participation of a wide range of groups including private landowners, agricultural organizations, local and regional government entities and other interested groups. Although participants agreed that there is no need or intention to undertake these programs immediately in Nebraska, it is important to initiate discussion now in order to facilitate the best possible planning for such possibilities in the future.

Detailed recommendations from each working group are presented below. In general, the consensus of those present at the meeting was that there is a great deal of work that needs to be done for this species in Nebraska and throughout its range. Research on the specific life history and habitat requirements for this species in Nebraska should be conducted as well as studies of

various land use management methods and the effect they have on orchid populations. It is essential that these studies be done collaboratively and cooperatively with other groups studying populations in other regions, using standardized methodology. This will enhance funding opportunities, significantly increase the value and applicability of the data and enable the most efficient utilization of resources to produce the maximum beneficial result. *Ex-situ* work such as genetics and *in-vitro* culture may also contribute significantly to our understanding of the biology of the species and to other issues critical to its survival.

SUMMARY OF RECOMMENDATIONS

Life History

1. Identify mycorrhizal species associated with the orchid at different sites and different life stages.

2. Determine number of seeds per capsule, seed viability (% and longevity) and germination rates in the field and in the laboratory using seed packet methods or other techniques (see: Caroline Hull-Sieg, Marge From).

3. Identify mechanisms and extent of seed dispersal in specific populations; correlate environmental factors with seed germination in the field using seed packets method (Caroline Hull-Sieg) and measurements of selected environmental factors (see Matrix)

4. Develop non-destructive methods including laboratory techniques to allow the following of below-ground stages (see Erica Szendrak and Marge From).

5. Conduct research to determine whether or not dormancy exists and, if so, what the trigger factors are and how long a plant can persist in this stage. Possible techniques to determine if plants (after absence/dormancy) are the same individuals when/if they reappear include:
    a. precise plotting of each individual occurrence
    b. genetic analysis (see Jennifer Hancock) of each individual occurrence
    c. radioactive (C14, etc.) tracer analysis
    d. deuterium tracer analysis
    e. explore portable non-invasive imaging or sensing systems

6. Encourage comparable data collection by developing (or utilizing an existing protocol) and distributing a standardized technical protocol for demographic monitoring which would include as a minimum detailed instructions for: a) tracking flowering plants for at least 10 years; and b) getting information on percentage of seed set. (Sather, Johnson, Hull-Sieg). Additional data on soil moisture, marking orchids and orchid locations should routinely be collected.

7. Gather comparable quantitative data on the number of flowering plants in current year that become absent, vegetative or reflower in the following year, seed production, number of leaves and the height of each plant for populations other than the Sheyenne, Pembina Trails,

Manitoba and other populations where this information already exists. Quantify factors related to cycling between flowering and non-flowering stages.

8. Conduct studies to identify the specific species of pollinators which are successful in a given area. These studies may involve direct observations on insects visiting inflorescences or light-trapping or sugar-baiting studies. Potential pollinators collected may then be examined for the presence of orchid pollinia ("a coherent mass of pollen", Dressler, 1990). General estimates of pollinator abundance and diversity would be useful management information.

9. Determine how the current year's environmental influences (especially micro-climate) affect growth and reproduction in the following year (Johnson, Sather, Hull-Sieg, Pleasants).

10. Maintain and continue established databases and develop new ones to answer the above questions.

Distribution

1. Select orchid sites in Nebraska for conservation actions based on the recommendations of the ecoregional planning documents being developed by The Nature Conservancy and the orchid recovery plan. Preserving these known, and currently unprotected populations, is the number one priority.

2. Document threats at individual orchid sites and incorporate this information into conservation planning and management documents.

3. Conduct county-based natural area inventories in eastern and central Nebraska to identify potential orchid habitats including tallgrass prairie, wet-mesic prairie, and wet meadows.

4. Standardize orchid survey methods.

5. Train biologist to conduct orchid surveys and develop a pool of capable surveyors.

6. Utilize and train volunteers to conduct orchid surveys where appropriate.

7. Conduct initial orchid surveys in the following priority habitats (see addendum 1): Sandhills wet meadows, concentrating on previously unsurveyed sites; Wet meadows in the lower Platte River Valley floodplain from Chapman eastward to the Missouri River; Tallgrass and wet-mesic habitats in Johnson, Pawnee, and Otoe counties in southeastern Nebraska; Wet meadows in Pierce County in northeast Nebraska; Wet meadows in the Elkhorn River floodplain from Basset southeastward to Valley.
8. Document site characteristics at orchid sites including soil texture and chemistry, associated vegetation, slope position, land use history, ground water hydrology, etc.

9. Secure funding to conduct orchid and county-based natural area inventories in Nebraska from: 1) private foundations, 2) Nebraska Environmental Trust, 3) USFWS Section 6 funds, 4) local government agencies, 5) private conservation organizations (including The Nature Conservancy's ecoregional planning efforts), and 6) other available funding sources.

Population Biology and Modeling

1. Initiate a seed bank study to determine the residence time of seeds in the soil.

2. Initiate a study to determine if a protocorm can directly become a flowering plant, bypassing a vegetative, aboveground stage.

3. Obtain estimate of number of seeds per mature capsule and determine whether "not so plump" capsules have any seeds (or viable seeds). Also determine variability in seed capsule production.

4. Begin plot studies of marked plants at several sites, especially upland prairie sites in an attempt to determine whether or not dormancy exists in this species. It will be necessary to follow plots in which all plants present are marked. Dormancy can be inferred when marked plants disappear for a period of years and then reappear (some researchers have noted a limit of 3 to 4 years on a plant returning from dormancy; Hull-Sieg, pers. comm.). The proportion of plants which disappear then later reappear needs to be determined as well as the length of the dormancy period, if possible. The group recognizes the limitations of this approach and, while precise plotting may not answer the dormancy question conclusively, the data it generates, along with the results of genetic studies, will significantly increase our understanding of this critical life stage issue.

5. Begin plot studies of marked plants at several sites, especially upland prairie sites, to study recruitment. It will be necessary to carefully examine plots every year to determine if new plants have appeared. The appearance of plants can be related to environmental variables such as precipitation.

6. Our demographic models to date are primarily for wet meadow, large population, sites at the northern end of the species range. Demographic data must be collected for upland prairie sites and populations in other parts of the species range. With regard to populations in other parts of the species range such as Nebraska, data from small sites, upland prairie populations and wet meadow populations in the sandhills would be most helpful with regards to furthering our understanding of this species.

7. Compare Sheyenne and Minnesota data sets. Take the raw data from both data sets and recast transition probabilities in the same way for both so they can be compared. Determine the impact of environmental variables on transition probabilities. Data collection efforts in these areas should be continued. The Sheyenne Model was based on a four-year data set, this in particular should be expanded to include more years. The impact of environmental variables and management treatment, and perturbations on transition probabilities and the sizes of different life stages need further examination and study.

8. See if the model can accommodate autocorrelations across years and across transition probabilities within years. Often wet years are followed by wet years and dry by dry. The cumulative effect of repeated conditions can be significant. Also, in the stochastic model some transition probabilities are affected similarly, i.e. if it is a bad year for flowering plants it should also be a bad year for vegetative plants.

Ex-situ Conservation

1. Develop effective means of plant propagation including protocols for germination and growth to maturity, with the goal of increasing the total number of plants.

2. Develop protocols for short and long term germplasm storage, such as seeds and protocorms.

3. Utilize *ex-situ* germination and culture studies to document developmental biology such as plant anatomy and physiology and to provide additional material for further research studies of genetics, morphology and longevity.

4. Undertake field studies of *in-situ* populations to compare developmental responses to *ex-situ* studies and to provide information such as soil requirements or mycorrhizal associations which may facilitate *ex-situ* cultivation.

5. Develop reintroduction techniques through controlled field research utilizing both direct and *in-vitro* transplants as well as direct seeding experiments.

6. Develop public display gardens, without disrupting wild populations, for public education. To reach a broad audience gardens could be developed at Nebraska Statewide Arboretums, zoos and state or federal parks.

7. Produce and distribute printed materials such as brochures, posters and interpretive signs for public education.

8. Develop education materials for use in school curricula introducing Great Plains rare and endangered species including the Western Prairie Fringed Orchid.

9. Recruit local nursery and garden centers and involve cooperative extension programs as an educational outlet to reach the general public.

Management

1. Determine the effects of hydrologic factors on management practices and address sources of hydrologic modifications including impacts of changes in groundwater levels on wet meadow habitat and impacts of changes in hydrologic processes on upland habitat.

2. Conduct research on current prairie ecosystem management practices and variables to determine their effects specific to the western prairie fringed orchid.
3. Determine effects of management practices on seasonal growth stages of the orchid.

4. Determine effects of climatic conditions in conjunction with management practices.

5. Integrate management and life history research when appropriate.

6. Develop and implement management plans using a short-term and long-term approach.

7. Use documented annual land use histories to structure and direct management research and devise short-term management.

8. Identify and address factors that may influence and limit the type and level of conservation and management practices that can be implemented. These include issues of land ownership and land use priority.

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Section 2
Life History Working Group Report

Life History Report and Recommendations

Goal: To insure the survival of the Western Prairie Fringed Orchid (*Platanthera praeclara*) as a species in the wild.

Objectives:

1. To identify crucial stages in the life cycle of the orchid and environmental influences which might affect them.
2. To generate and prioritize recommendations on needed research and management to achieve the above objectives.

Process/Influence Matrix:

Participants spent the first working group session brainstorming regarding issues of importance to the life history of the western prairie fringed orchid. Twenty-five issues were identified and each was then classified as either a 'life process' or an 'influence' and sorted according to its relevance to each of 5 life-stage categories (seed, protocorm/seedling, vegetative or non-flowering, flowering and dormant). The next step was to construct a matrix for each life-stage in an effort to determine the importance (Yes or No) of the identified influences on the life processes and the level information available (none, low, medium, high) regarding the effect of the particular influence on the life process. This information is presented in matrices 1 through 4. Where information is available, the source (author) of the information is identified. We used information presented in the matrices to generate and prioritize needed research and management recommendations.

Seed Stage:
Importance
Available Knowledge

Process

Influence	Viability	Germination	Dispersal
Soil Temp	Yes None)	Yes L	No --
Soil properties	Yes None	Yes None (Hull-Sieg-- Mg)	No --
Hydrology/ Flood	Yes None	Yes None)	Yes L (Hull-Sieg)
Predation	No --	No --	Yes None
Disease	Yes None	Yes None	No --
Management techniques	Yes None	Yes None	Yes None

Length of stage – unknown; Per Anu Sud, CPC:--- 3-5 years to collect!

Protocorm:
Importance
Available Knowledge

Process

Influence	Length of Stage	Mycorrhizal infection	Root distribution	Mortality
Soil properties	Yes None	Yes L (Zelmar)	Yes None	Yes None
Hydrology	Yes L (Hull-Sieg)	??? ????	Yes L (Johnson, Hull-Sieg)	Yes L (Pleasants, Hull-Sieg)
Disease	Yes None	Yes None	Yes None	Yes None
Predation	Yes None	No --	Yes None	Yes None
Competition/ Allelopathy	Yes None	Yes None	Yes None	Yes None
Management techniques	Yes None	Yes None	Yes None	Yes None
Soil temperature	Yes None (Hull-Sieg)	Yes None	Yes None	Yes None

Vegetative Stage (Non-flowering):
Importance
Available Knowledge

Process

Influence	Length of Stage	Adult Cycle	Root Distribution	Mycorrhizal association	Energy Reserves
Predation	Yes None	Yes None	???? None	Yes None	Yes None
Competition	Yes None	Yes None	Yes None	Yes None	Yes None
Hydrology	Yes None	Yes None)	Yes Low	Yes None	Yes None
Soil properties	???? None	??? None	Yes L (Johnson)	Yes L (Literature)	Yes L (Hull-Sieg)
Soil Temp.	Yes None	??? None	Yes None	Yes None	Yes None
Air Temp.	Yes L (Johnson)	Yes L (Johnson)	No --	No --	Yes L (Johnson)
Management techniques	Yes L (Pleasants, Hull-Sieg)	Yes L (Pleasants, Hull-Sieg)	Yes None	Yes None	Yes L (Pleasants)
Land Use	Yes None	Yes None	No --	No --	Yes None

Note: Previous year's environmental variables affect current year's vegetative state.

Flowering Stage:
Importance
Available Knowledge

Process

Influence	Length of stage	Phenology	Pollination	Population flowering patterns	Energy reserves (reproduction)	Root Distribution	Mycorrhizal association	Seed production
Population Isolation	No --	No --	Yes None	No --	No --	No --	??? None	Yes L (Ashley)
Competition	??? None	Yes None	No --	???? None	Yes None	Yes None	No --	No (Johnson) --
Pollinators	No --	No --	Yes H	No --	No --	No --	No --	Yes H
Soil properties	No --	No --	No --	Yes None	Yes --	Yes None	Yes None	Yes None
Soil Temp	Yes L (Henszey)	Yes? None	No --	No --	Yes None	No --	Yes None	No --
Hydrology	Yes L(Hull-Sieg, Henszey, Johnson)	Yes (Johnson)	No --	Yes None	Yes None	Yes None	Yes None	Yes L (Hull-Sieg, Sather, Johnson)
Air Temp (degree days, killing frost)	Yes L (Pleasants)	Yes L (Hull-Sieg, Pleasants)	Yes None	No --	Yes None	No --	No --	Yes None
Management techniques	Yes L (Hull-Sieg, Pleasants, Currier)	Yes L (Hull-Sieg, Pleasants)	Yes None	Yes None	Yes None	Yes None	Yes None	Yes None
Land Use	Yes None	Yes None	Yes None	No --	Yes None	Yes None	No --	Yes None
Predation	Yes None	Yes None	Yes None	No --	Yes None	Yes None	No --	Yes None
Disease	Yes None	Yes None	No --	No --	Yes None	Yes None	No --	Yes None

Life History
Stage-based Information and Recommendations

Seed Stage:
The Western Prairie Fringed Orchid reproduces almost exclusively (99%) by seed, so production, dispersal, viability and germination are crucial to survival of the species. We lack knowledge on all of the above processes and how they are affected by environmental factors, disease and management. Results of the modeling suggest that variables in the seed stage most critically effect survival of the population. Therefore, this stage and the recommendations outlined below were identified as top priorities for research. The recommendations reflect important information needs.

Recommendations:

1. Determine seed viability (% and longevity) and germination rates in the field and in the laboratory using seed packet methods or other techniques (see: Caroline Hull-Sieg, Marge From).

2. Try to correlate environmental factors with seed germination in the field using seed packets method (Caroline Hull-Sieg) and measurements of selected environmental factors (see Matrix)

3. Identify mechanisms and extent of seed dispersal in specific populations.

Protocorm/Seeding Stage:
Although the protocorm/seedling stage is an important stage in the life cycle of this species as a link (stage) between seed and the more advanced stages (vegetative and flowering), the inherent ability to document this below ground stage makes identification of specific recommendations difficult. Therefore, it is important that methods be developed to allow us to more fully document this stage, particularly research related to root distribution, mycorrhizal associations, and land management practices.

Recommendation:

1. Develop non-destructive methods including laboratory techniques to allow the following of below-ground stages (see Erica Szendrak and Marge From).

Vegetative Stage:
This stage is important because it includes most (75-90%) of the above-ground plants in a population. It can both precede and succeed the flowering stage in any individual plant because it is composed of below ground seedling and dormant stages coming above the ground and, potentially, flowering plants which have reduced energy reserves but are still able to maintain an above-ground presence.

Recommendation:
1. Quantify factors related to cycling between flowering and non-flowering stages.

Flowering Stage:
The flowering stage is the most visible stage in the life history of this species. Many abiotic factors may affect the production of flowers but few empirical studies to determine this relationship have been conducted. Pollination of flowers usually results in the production of viable seeds. Indirect evidence of successful pollination would be the occurrence of inflated seed pods containing viable seeds. However, it is still important to determine the identity and abundance of potential and actual pollinators present in areas where *P. praeclara* is known to occur. It is usually assumed that species of long-tongued hawkmoths (*Spingidae*) are the natural pollinators of this orchid. Unfortunately, little published data exists which documents the species of moths associated with *P. praeclara* pollination. It is often not known, in orchid populations showing low seed production whether appropriate pollinators are present.

Adult hawkmoths may be generalists in their choice of flowers visitation, however, larval hawkmoths (caterpillars) are often very specific in their choice of food plant. These food plants are often species of plants found in the area [i.e. grapevine, hackberry, etc.] on which hawkmoth eggs are deposited. Land managers should insure that host plants for hawkmoth caterpillars are present near orchid populations.

Our current understanding of the biology of *Platanthera praeclara* is primarily based on information derived from field studies on a limited number of populations located at the northern edge of the species distribution. It is appropriate to expand our information base to include data obtained from populations throughout the geographic range of the species.

Recommendations:

1. Gather comparable quantitative data on the number of flowering plants in current year that become absent, vegetative or reflower in the following year, seed production, number of leaves and the height of each plant for populations other than the Sheyenne, Pembina Trails, Manitoba and other populations where this information already exists.

2. Conduct research to accurately determine the number of seeds per pod. (Marge From)

3. Conduct studies to identify the specific species of pollinators which are successful in a given area. These studies may involve direct observations on insects visiting inflorescences or light-trapping or sugar-baiting studies. Potential pollinators collected may then be examined for the presence of orchid pollinia. General estimates of pollinator abundance and diversity would be useful management information (David Ashley).

Dormancy Stage:
Due to the uncertainty regarding the existence of dormancy in this species, accurate identification of individuals is of primary importance. Upon determination of dormancy, thorough research into this life stage will be required.
Recommendations:

1. Conduct research to determine whether or not dormancy exists and, if so, how long a plant can persist in this stage. Possible techniques to determine if plants (after absence/dormancy) are the same individuals when/if they reappear include:
    a. precise plotting of each individual occurrence
    b. genetic analysis (see Jennifer Hancock) of each individual occurrence
    c. radioactive (C14, etc.) tracer analysis
    d. deuterium tracer analysis
    e. portable MRI or similar non-invasive imaging or sensing systems.

2. Identify dormancy trigger factors. Identify whether or not dormancy is related to depletion of energy reserves, or to environmental factors.

General Life History Recommendations:
1. Determine how the current year's environmental influences (especially micro-climate) affect growth and reproduction in the following year (Johnson, Sather, Hull-Sieg, Pleasants).

2. Encourage comparable data collection by developing (or utilizing an existing protocol) and distributing a standardized technical protocol for demographic monitoring which would include, as a minimum, detailed instructions for (Johnson, Sather, Hull-Sieg):
    1. tracking flowering and vegetative plants for at least 10 years;
    2. getting information on percentage of seed set; and
    3. measuring soil moisture.

Working Group Members: Dave Ashley, Onnie Byers, facilitator, Mike Fritz, Jennifer Hancock, Bob Henszey, Karen Johnson, Tim Knott, Ed Louis, Ed Plotka, Bud Reese, Gary Willson

POPULATION AND HABITAT VIABILITY ASSESSMENT (PHVA) FOR THE WESTERN PRAIRIE FRINGED ORCHID (*Platanthera praeclara*)

Eugene Mahoney State Park
Ashland, Nebraska
27-30 April 1997

Final Report

Section 3
Distribution Working Group Report

Distribution and Field Survey Report and Recommendations

This working group dealt primarily with the Nebraska populations of western prairie fringed orchid and covered the topics of distribution, surveys, threats, and conservation priorities.

Distribution

The historic distribution of the western prairie fringed orchid (*Platanthera praeclara*), based on collection records, includes eastern Nebraska, much of central Nebraska and the Sandhills (Figure 1). Presently the orchid is known from 14 sites in eastern and central Nebraska (Figure 1). Within the Central Tallgrass Ecoregion of Nebraska (eastern fourth of the state) the orchid is found on lower slopes and ravines of upland tallgrass prairie and on wet-mesic prairies within ravines and riparian areas. Within the Central Tallgrass Prairie Ecoregion, one site is known from the Platte River Valley in Hall County in central Nebraska and several populations are known from wet meadows in the Sandhills of Cherry County. Site specific data for these populations is presented in Table 1. The total range of the orchid includes much of the Midwest (Figure 2, range map from Recovery Plan). this information is not included in this report, but is available in the Western Prairie Fringed Orchid Recovery Plan and from state heritage programs.

Surveys for the western prairie fringed orchid in Nebraska have been limited. Systematic surveys have been conducted by the U.S. Fish and Wildlife Service on portions of the Valentine National Wildlife Refuge in Cherry County and by the Nebraska Public Power District on portions of the central and western Platte River Valley. The majority of central and eastern Nebraska have not been inventoried for the orchid. Factors complicating completion of the surveys includes lack of knowledge on the location of the large majority of tallgrass prairies and wet meadows in eastern Nebraska. County-based natural area inventories have not been completed in Nebraska. In addition, the Sandhills contain over a million acres of unsurveyed wet meadow habitat. Lack of funding sources is a primary limiting factor for why orchid surveys and natural area surveys in general have not been conducted in Nebraska.

Table I. Western prairie fringed orchid (*Platanthera praeclara*) in Nebraska (modified from the orchid recovery plan) Observations through 1997

County	Site name	pop.[1]	Protection level	Eco Region[2]	Ownership	1st Seen	Last seen	Predominant management	Monitoring
Cherry	Valentine NWR H.U. 32B2	77 105 (1994)	6	CMP	USFWS	1990	1997	Rest	Annual census
Cherry	Valentine NWR	123?	6	CMP	USFWS	1994?	1997	Rest	Annual census
Cherry	Valentine NWR	2?	6	CMP	USFWS	1979	1997	Rest	Annual census
Cherry	Road side park	2	0	CMP	Private	1993	1993	Grazed/Hayed/	Periodic census
Cherry	CNW-Arabia	22	0	CMP	Private	1985	1997	Hayed	
Cherry	Watts Lake	21 35 (1996)	0	CMP	Private	1993	1997	Hayed	Annual census
Cherry	Steverson Lake WMA	8	8	CMP	NE Game & Parks	1996	1997	Hayed	Periodic census
Cherry	Lord Meadow	1	0		Private	1997	1997	Hayed	?
Cherry	Duck Lake	11 (1993)	0	CMP	Private	1986	1997	Rest	Annual census
Hall	Mormon Island Crane meadow	60	8	CMP	WC Trust	1978	1997	Grazed/Burned	Periodic census
Lancaster	Livengood Prairie	2	0		Private	1997	1997	Hayed	?
Lancaster	Nine Mile Prairie	176	8	CTP	NE	1984	1997	Burned	Demographic
Otoe	Dicken Prairie	32	0	CTP	Private	1995	1997	Hayed	
Otoe	East Elberon Prairie	2	0		Private	1997	1997	Hayed	?
Otoe	West Elberon Prairie	1	0		Private	1997	1997	Hayed	?
Pierce	Radek Prairie	5	0	CTP	Private	1996	1996	Hayed	Periodic census
Pierce	Zimmerman Meadow - NE	60	0		Private	1997	1997	Hayed	?
Pierce	Zimmerman Meadow - SW	35	0		Private	1997	1997	Hayed	?
Sarpy	Krebs Prairie	60	2	CTP	Private	1993	1997	Hayed	Periodic census
Saline	Kubacek Prairie	15	0	CTP	Private	1996	1997	Hayed	Periodic census
Seward	Twin Lakes WMA	50	8	CTP	NE Game& Parks	1982	1997	Burned	

[1] Highest population level known from survey period .
[2] CTP - Central Tallgrass Ecoregion, CMP - Central Mixedgrass Ecoregion.

Threats

The orchid recovery plan discusses in detail threats and limiting factors for orchid populations and habitats over the entire range of the orchid. The plan also discusses conservation measures for the species in detail. Threats and limiting factors have not been documented at all Nebraska orchid sites. This information is needed to prioritize conservation and management efforts. Potential threats to the orchid and orchid habitats in Nebraska are listed below:

1) Urban development in counties containing major metropolitan areas.
2) Conversion of native prairies to agricultural lands.
3) Pesticide (both herbicide and insecticide) use (which can also impact pollinator populations).
4) Ditching of wet meadows in the Sandhills and river floodplains which alters ground water hydrology.
5) Invasion of orchid habitats by exotics plants including Canada thistle (*Cirsium canadensis),* leafy spurge (*Euphorbia esula),* smooth brome (*Bromus inermis*), Kentucky bluegrass (*Poa pratensis*), timothy (*Phleum pratensis* spp), and reed canary grass (*Phalaris arundinacea*).
6) Alteration of natural stream flows which alters meadow hydrology.
7) Sand and gravel mining along the Platte, Elkhorn and Loup Rivers.
8) Improper use of land use practices including haying and grazing (lack of management on protected sites can lead to litter build up).
9) Lack of fire which can lead to litter build up, and woody encroachment and exotic invasions.
10) Over collecting and trampling by sight seers.

Conservation Priorities

The lack of surveys and limited knowledge on the distribution and abundance of the orchid has hindered setting conservation priorities and protection efforts for the species in Nebraska. Setting conservation priorities for the orchid solely for Nebraska has limited value due to the recent trend towards ecoregional land conservation efforts. The Nature Conservancy is presently conducting ecoregional planning for the Central Tallgrass Ecoregion. They likely will begin this same process for the Central Mixedgrass Prairie ecoregion within the next year. Conservation priorities and methods for protecting the orchid in Nebraska should follow the recommendations established.

Recommendations

1) Select orchid sites in Nebraska for conservation actions based on the recommendations of the ecoregional planning documents being developed by The Nature Conservancy and the orchid recovery plan. Preserving these known, and currently unprotected populations, is the number one priority.

2) Document threats at individual orchid sites and incorporate this information into conservation planning and management documents.

3) Conduct county-based natural area inventories in eastern and central Nebraska to identify potential orchid habitats including tallgrass prairie, wet-mesic prairie, and wet meadows.

4) Standardize orchid survey methods.

5) Train biologist to conduct orchid surveys and develop a pool of capable surveyors.

6) Utilize and train volunteers to conduct orchid surveys where appropriate.

7) Conduct initial orchid surveys in the following priority habitats (see addendum 1):

   a) Sandhills wet meadows, concentrating on previously unsurveyed sites.
   b) Wet meadows in the lower Platte River Valley floodplain from Chapman eastward to the Missouri River.
   c) Tallgrass and wet-mesic habitats in Johnson, Pawnee, and Otoe counties in southeastern Nebraska.
   d) Wet meadows in Pierce County in northeast Nebraska.
   e) Wet meadows in the Elkhorn River floodplain from Basset southeastward to Valley.

8) Document site characteristics at orchid sites including soil texture and chemistry, associated vegetation, slope position, land use history, ground water hydrology, etc.

9) Secure funding to conduct orchid and county-based natural area inventories in Nebraska from: 1) private foundations, 2) Nebraska Environmental Trust, 3) USFWS Section 6 funds, 4) local government agencies, 5) private conservation organizations (including The Nature Conservancy's ecoregional planning efforts), and 6) other available funding sources.

Working group members: Mark Dietz, Wally Jobman, Leonard McDaniel, Jeff Peake, John Pearson, Glenn Pollock, Gerry Steinauer.

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Section 4
Population Biology and Modeling Working Group Report

Population Biology and Simulation Modeling of the Western Prairie Fringed Orchid (*Platanthera praeclara*): Working Group Report

Introduction: The Value of Constructing Orchid Population Models

Constructing a model involves both a process and a product. Both can be of significant value, and in some cases—perhaps this one in particular—the process may be more important than the product. The process of constructing these models required the working group participants to think carefully about the biology of the species and evaluate the data available in order to determine quantitative characteristics of the species' life history. It also revealed the portions of the life cycle that we know little or nothing about and which warrant further investigation. Additionally, an important outcome of this process was a clarification of the issue of dormancy in the species. Putting together the data sets from North Dakota's Sheyenne National Grassland and from several sites within the Minnesota Pembina Trail metapopulation was also very instructive. This comparison had not been made prior to this workshop and it revealed some significant demographic differences between populations in the two areas.

The models presented and discussed in this section were developed to chart the stage-based life cycle of the western prairie fringed orchid *Platanthera praeclara.* Insight gained through this iterative modeling process is vital for a better understanding of the population dynamics of the species. The goals of the process are to explore issues such as the clarification of our collective understanding of the species' life history, the prediction of future orchid population sizes under different environmental conditions and management regimes, the dynamics among different populations in different study sites, and the identification of specific information gaps and resultant research needs.

A basic stage-based stochastic model for population dynamics of the western prairie fringed orchid was developed using the RAMAS/Stage software package (Ferson, 1994). Stage-based models have been shown to be extremely useful in the analysis of species for which the primary unit of population organization is the developmental stage of the individual and not necessarily its age (Ferson, 1994; Lefkovitch, 1965). This characteristic is very common among plants, where individuals may be of the same chronological age but may occupy radically different developmental stages of the species' life history, i.e., seedling, vegetative plant, or flowering plant. The model's fundamental algorithm describing the growth of the population is composed of a series of equations (called replacement functions) relating the abundances within individual stages at time $t+1$ to the abundances of those stages at time t. These functions include transition probabilities, or estimates of the probability of individuals in stage i in year t either remaining in that stage or developing into stage j at time $t+1$.

Stage Definitions

All of our models incorporate the following generalized stages:

Seeds - Viable seeds that are produced by flowering plants. This includes all seeds contained in a seed bank, if such a bank indeed exists;
Protocorm and Seedlings - The initial growth of the seed to aboveground growth;
Nonflowering Plants - Plants with vegetative aboveground growth only, no flowers;
Flowering Plants - Plants with flowers present and visible;

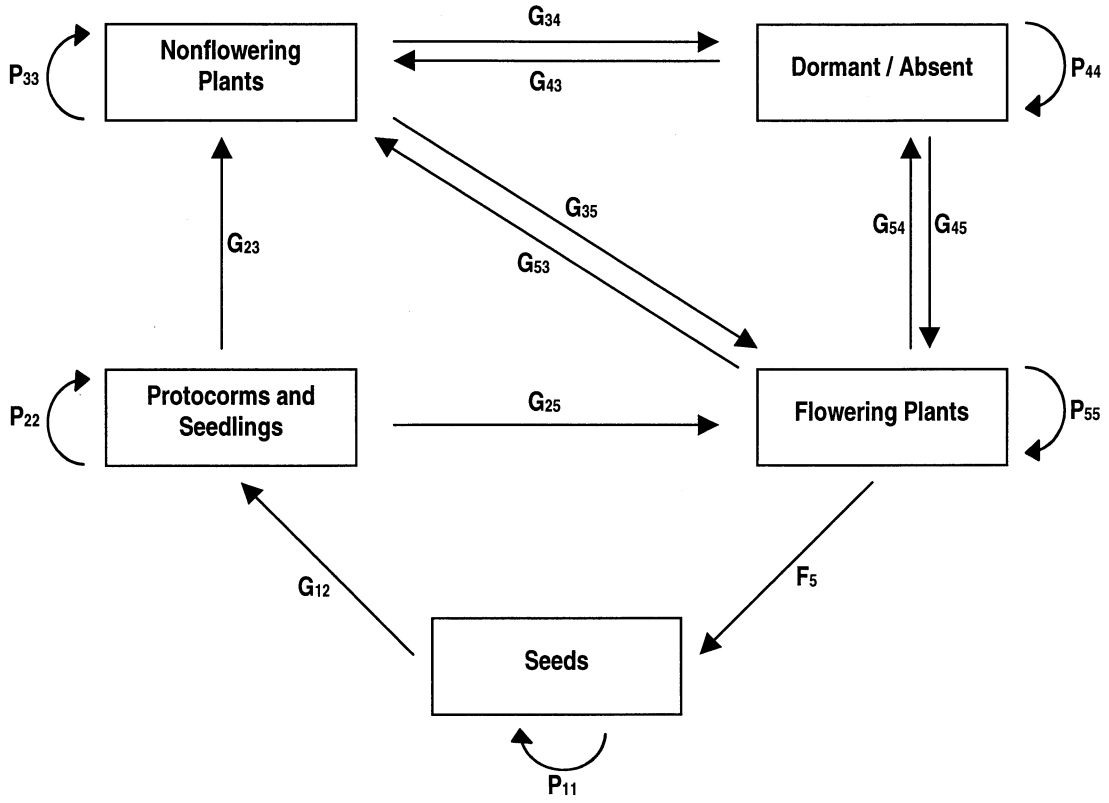
Dormant/Absent Plants - Plants that once had aboveground growth, but have returned to a state below ground or, in the case of the Sheyenne National Grassland model (see below), may have died.

The basic model employs within- and between-stage transition probability estimates based on Sheyenne National Grassland data from Sieg and King (1995), and unpublished Minnesota data from Sather and Menges & Quintana-Ascencio for aboveground stages, and "ballpark" best guesses as well as unpublished preliminary data for numbers of seeds and transition probabilities from seeds to aboveground stages. The aboveground transition probabilities are based on four years of data from the Sheyenne National Grassland and 12 years of data from Minnesota, collected between the years of 1990-1994 and 1985-1996 respectively.

The stage-based model can be described in a number of ways, including a graphical depiction of the transitions observed both within and between stages, a traditional matrix view showing the stage-transition matrix (also known as a Lefkovitch matrix) of transition probabilities, and by a series of equations relating the abundance in a given stage in year $t+1$ to the abundances in all other stages in year t. Refer to Figure 1 in this section for the network and a depiction of the transition probabilities within a given stage (labeled P_{ii}) and the transitions between stage i and stage j (labeled G_{ij}).

Differences between the Minnesota and North Dakota data sets relating to the reporting of dead and dormant plants led to the basic model being expanded into two closely related baseline models, the *Sheyenne* model and the Minnesota model. In the Sheyenne model, death may occur only from the Dormant/Absent Plant stage. Flowering and nonflowering plants can only die by moving through the Dormant/Absent Plant stage. In contrast, the Minnesota model limits the Dormant/Absent Plant stage to plants that will subsequently appear above ground. Thus deaths in this model can occur in the Flowering Plant and Nonflowering Plant stages, but not in Dormant/Absent Plant stage.

Figure 1. Generalized stage-based population dynamics model for the western prairie fringed orchid (*Platanthera praeclara*). The model is shown as both a graphical network (top) and a standard projection matrix (bottom).



	Present status				
Status next year	**Seeds**	**Protocorm**	**Nonflowering**	**Dormant**	**Flowering**
Seeds	P_{11}				F_5
Protocorm	G_{12}	P_{22}			
Nonflowering		G_{23}	P_{33}	G_{43}	G_{53}
Dormant			G_{34}	P_{44}	G_{54}
Flowering		G_{25}	G_{35}	G_{45}	P_{55}

The initial models introduced above began as strictly deterministic in nature; in other words, transition probabilities were fixed and did not vary stochastically over time. These initial models were subsequently modified to account for stochastic variation estimated from the published data sets of Sieg and King (1995) and unpublished data sets of Sather, Pleasants and Menges & Quintana-Ascencio. All of the estimates of environmental variation are described statistically as lognormal distributions, with the specified means and standard deviations obtained directly from the temporal datasets mentioned above. All models were initiated with 100 flowering plants, with estimates for the abundances in the remaining stages based on this initial value. Model projections were run out to 50 years.

Sheyenne National Grassland Model

Data were collected from 16 sites on the Sheyenne National Grassland, which included a variety of management treatments involving varying combinations of grazing and burning. Table 3 in Sieg and King (1995) was the primary source of information in developing aboveground transition probabilities.

The equations describing these transitions are listed below, where the numerical abundance within a given stage is depicted as [stage]:

[seeds] = 27300[flowering] + 0.5[seed]
[protocorm] = 0.0056[seeds]
[non-flowering] = 0.0075[protocorm] + 0.29[non-flowering] + 0.2425[flowering] + 0.0967[dormant]
[dormant] = 0.5475[non-flowering] + 0.59[flowering] + 0.15[dormant]
[flowering] = 0.1625[non-flowering] + 0.0267[dormant] + 0.00248[protocorm] + 0.1675[flowering]

As an example, the number of seeds present in a given population in year t +1 is therefore given by the total seed production by all flowering plants present in year t and by the number of seeds present in year t that have remained viable through to the next year in the seed bank. Furthermore, it is estimated that the germination rate for these seeds is 0.56%.

The justification for these values is described in greater detail below.

Seeds

The initial number of seeds was based on the assumption that each *P. praeclara* seed pod produces 27,300 viable seeds. This estimate was based on Margaret From's unpublished laboratory observations of about 500 seeds on a standard laboratory culture plate on a total of 200 plates, totaling 100,000 seeds. This was judged to be about half of the production from 6 pods; therefore, total production per pod would be approximately 30,000.

To obtain the number of viable seeds produced by a flowering plant, we assumed that, on the average, a plant had 7 flowers of which 20% produced pods (Pleasants and Moe, 1993). We estimated that 65% of the seeds in these pods were viable, again based on unpublished data from Margaret From. Therefore

Viable seeds per flowering plant (F_5) = 30,000(7)(0.2)(0.65) = 27,300

Additional models were developed that reduced viable seed production to 0.75 and 0.5 of this baseline value (F_5 = 20,475 and 13,650 respectively).

We estimated that half of the seeds remained in the seed bank annually, thus the probability of a seed in year t remaining as a seed in year t +1 was 0.5. The possibility of a seed bank was based on observations from Sheyenne National Grassland of plants appearing in study plots in areas where no flowering plants had been seen for 3 or more years. Some members of the working group felt that this was too long a period for these newcomers to be plants which returned from dormancy and that some of them had come from a seed bank. As a guess, 50% of the seed was assumed to remain viable from one year to the next. Very little is known about the longevity of orchid seeds in the soil. To investigate the influence of this seed bank on the dynamics of prairie

fringed orchid populations, additional models were run that reduced seed residency to 0.25, 0.125 and 0.0 (i.e., no seed bank present).

Protocorms and seedlings

The probability estimates for a seed germinating to become a protocorm were based on very preliminary unpublished data from a study site in the Sheyenne National Grassland in 1996. A total of 36 experimental seed packets placed in natural conditions yielded a total of 4 seedlings. The amount of seeds per packet by weight was set at 0.001 mg, but the number of seeds per packet was unknown. Therefore, a guess of the number of seeds per packet was set at 500. In addition, as the data analysis is not yet complete, the total number of protocorms produced in this trial has not yet been determined; therefore, we estimated a total of 100 protocorms. Therefore, the probabilities for the underground transitions were calculated as follows:

$$\text{Seed - Protocorm } (G_{12}) = (100) / \{(36)(500)\} = 0.0056$$

Additional models with $G_{12} = 0.0042$ and 0.0028 were developed to investigate the response of an orchid population to reduced germination rates.

Aboveground

The probability of a protocorm becoming an aboveground individual was based on the observation (see above) of 4 seedlings relative to 100 protocorms, and assuming that 25% of the seedlings would survive. Based on data in Table 2 in Sieg and King 1995, the percentage of new plants that flower (i.e. plants that first appear above ground as flowering plants) averages 24.8%, compared to 75.2% that are vegetative. Further, we guessed that 25% of these seedlings would survive to an aboveground stage.

$$\text{Protocorm - Nonflowering } (G_{23}) = (4/100)(0.752)(0.25) = 0.00752$$

$$\text{Protocorm - Flowering } (G_{25}) = (4/100)(0.248)(0.25) = 0.00248$$

As a result of considerable discussion among the group members concerning the possibility of a direct transition from protocorm to flowering plant, an alternative model was developed within which this particular transition was removed.

The remaining transition rates are based on data in Table 3 of Sieg and King (1995). The within-dormancy transition P_{44} is calculated as 1 - [average probability of a plant absent in year t remaining absent in year $(t + 1)$]. Additionally, a set of models was developed from the data of Sieg and King (1995) that alternatively represented "good" and "bad" conditions for orchid population growth. Most of the aboveground stage transitions were modified based on extreme values, representing good and bad years, observed in the original dataset. These transitions are tabulated below.

	Conditions		
Transition	Baseline	"Good"	"Bad"
P_{33}	0.29	0.455	0.125
G_{34}	0.5475	0.29	0.805
G_{35}	0.1625	0.255	0.07
G_{53}	0.2425	0.355	0.136
G_{54}	0.59	0.40	0.77
P_{55}	0.1675	0.245	0.094

The Minnesota Model

The Minnesota model uses transition probabilities obtained from Sather's data set (unpublished but made available during the workshop) from several sites near the Pembina Trail in northwestern Minnesota. The seed production and belowground transition probabilities for the Minnesota model are the same as for the Sheyenne model; in addition, the perturbations described in the previous section were also applied to these models. One difference to note is that because of the nature of the summary tables available to us, we had to treat the dormant stage differently than for the Sheyenne data set. In particular, we define the dormant stage for the Minnesota data set as "plants that are alive, but underground". Defined as such, plants in this stage are not subject to death; they will emerge above ground at some later time. For the Sheyenne data set, the dormant stage is defined as plants that became absent and whose future status includes the possibility of death or returning above ground, the latter representing true dormancy. Because of the difference in the definition of "dormant" the values for the transition probabilities of entering and exiting the dormant stage cannot be compared between the 2 models, but the values for all other transitions can be compared. The key difference then is that in the Sheyenne model plants can only enter the death stage, which is not explicitly included in the model, via the dormancy stage (this is referred to in our group as "death by dormancy"); in the Minnesota model plants can only enter the death stage from the non-flowering stage and the flowering stage (referred to as "death by any means").

The transition probabilities we used were obtained as follows (**Note:** Tables in this sub-section refer to those found in the unpublished manuscript by E. S. Menges and P. F. Quintana-Ascencio which summarizes Sather's Minnesota data set):

<u>Non-flowering stage</u>

Non-flowering - Dormant (G_{34}) 0.137

(From Table 8, the average proportion of non-flowering plants becoming dormant)

Non-flowering - dead 0.139

(From Table 4, the average annual mortality rate for aboveground plants. The data in table 4 is for all aboveground plants combined. The table does not provide separate mortality rates for vegetative and flowering plants. Consequently, we used the average aboveground mortality rate for both vegetative and flowering plants.)

Of the remaining proportion of plants in this class (0.724), Table 7a indicates that 0.789 of these plants remain non-flowering and 0.211 become flowering. Therefore:

Non-flowering - Non-flowering (P_{33}) 0.571 {(0.724)(0.789)}

Non-flowering - Flowering (G_{35}) 0.153 {(0.724)(0.211)}

Flowering Stage

Flowering - Dormant (G_{54}) 0.102
(From Table 8, the average proportion of flowering plants becoming dormant)

Flowering - Dead 0.139
(From Table 4, the average annual mortality rate for aboveground plants)

Of the remaining proportion of plants in this class (0.759), Table 7a indicates that 0.58 of these plants remain flowering and 0.42 become non-flowering. Therefore:

Flowering - Non-flowering (G_{53}) 0.319 {(0.759)(0.42)}

Flowering - Flowering (P_{55}) 0.440 {(0.759)(0.58)}

Dormant Stage

These are plants that are operationally defined as still alive and which will come back at some point in time. This class includes plants that have been dormant for 1-9 years. The data set does not provide the probability of an average dormant plant returning to aboveground status. Instead we have information on the163 plants that were dormant during the study proportion (Table 5). This data indicate what proportion were dormant for 1, 2, etc. years. This tells us that any plant that has been in the dormant pool for 1 year has a 69% probability of reemerging the next year (112/163), any plant that has been in for 2 years has a 47% chance of reemerging the next year (24/(163-112)) etc. To calculate the probability of an *average* dormant plant reemerging we need to know the proportion of dormant plants that are in each age group and weight their probability of reemerging by this proportion. This equals: ((prop. plants 1 yr. old) x (prob. of 1 yr. old plants reemerging)) + ((prop. 2 yr. old) x (prob. of 2 year old plants reemerging)) and so on. The problem lies in determining the proportion of plants in each age class. To obtain this we assume that when the population achieves a stable stage distribution, which does occur in a deterministic model such as those run here initially, the age distribution of the dormant pool will mirror the frequency distribution in Table 5. On this basis we calculate that 57% of plants will leave the dormant pool every year, leaving 43% remaining in the pool.

Dormant - Dormant (P_{44}) 0.43
(From Table 5, as described above)

Dormant - Dead Not applicable by definition

57% of plants in the dormant pool reemerge. Of this group that returns above ground, 90% come back as vegetative and 10% as flowering (Table 7a):

Dormant - Non-flowering (G_{43}) 0.513 {(0.57)(0.9)}

Dormant - Flowering (G_{45}) 0.057 {(0.57)(0.1)}

Unfortunately, there was no marked variation in mortality rate among years for the Minnesota populations so consequently we did not construct "good" and "bad" environment model sets such as those for the Sheyenne National Grassland dataset.

Comparison of Transition Probabilities between the Sheyenne and Minnesota Models
The primary difference between the two population models described above is that plants in the Sheyenne National Grassland show much higher rates of mortality. For the Sheyenne model the proportion of non-flowering plants that go to the dormant class is 0.5475; for the majority of these individuals, this transition will ultimately result in death. In the Minnesota model the proportion of non-flowering plants dying is only 0.139. A similar disparity can be seen for flowering plants where 0.59 become absent (≈ death) at Sheyenne while only 0.14 die in Minnesota. The major reason for this difference is that the Sheyenne data set includes 2 years of very high disappearance rates corresponding to one drought year (1990-1991) and one flood year (1993-1994). Disappearance rates for vegetative plants at Sheyenne over the 4 years of the study reported (Table 5, Sieg and King 1995) were 0.76, 0.26, 0.32, and 0.85; these approximately equal death rates. For the Minnesota plants over the same period the mortality rates were 0.176, 0.119, 0.118, and 0.031 (Table 4). For the Minnesota data if we add the plants that went dormant to produce a disappearance group, the numbers are 0.368, 0.298, 0.428, and 0.160. Not only are the Minnesota values markedly lower, but the extent of variation, even over the same four years of observation, is also markedly lower. Discussions during the workshop led to the conclusion that, perhaps due to differences between the two areas in topography and hydrology, the Minnesota populations appear to be more effectively buffered against the effects of precipitation extremes than are the Sheyenne populations.

A summary of the baseline model parameters for the alternative sets of models is presented in the two standard projection matrices below.

Sheyenne National Grassland

	Present status				
Status next year	**Seeds**	**Protocorm**	**Nonflowering**	**Dormant**	**Flowering**
Seeds	0.5				27300.0
Protocorm	0.0056				
Nonflowering		0.0075	0.2900	0.0967	0.2425
Dormant			0.5475	0.1200	0.5900
Flowering		0.0025	0.1625	0.0267	0.1675

Minnesota

	Present status				
Status next year	**Seeds**	**Protocorm**	**Nonflowering**	**Dormant**	**Flowering**
Seeds	0.5				27300.0
Protocorm	0.0056				
Nonflowering		0.0075	0.5710	0.5130	0.3190
Dormant			0.1370	0.4300	0.1020
Flowering		0.0025	0.1530	0.0570	0.4400

Model Results

Deterministic Model Results

Sheyenne National Grassland

The long-term deterministic population growth rate, or λ, can be calculated using standard life-table analysis. These calculations assume that there is no annual variation in birth and death rates (resulting from either random environmental fluctuations or from stochastic demographic variation), that the availability of mates is never a limiting factor, and that the stage-specific abundances are at an equilibrium, stable state. Populations with $\lambda > 1.0$ are increasing over time while those with $\lambda < 1$ are decreasing over time. Given these assumptions, and our best estimates of the life-history parameters for this population, the Sheyenne National Grassland baseline model shows the capacity for considerable annual growth with $\lambda = 1.135$ (Table 1). In other words, this simulated population is expected to grow, in the absence of stochastic variation in vital rates, at a rate of 13.5% per year.

Table 1. Western prairie fringed orchid (*Platanthera praeclara*) population viability. Output for the deterministic and stochastic Sheyenne National Grassland models under baseline conditions (see the text for a description of these conditions). The population growth rate per generation is given by λ, while P(E) is the probability of population extinction within the 50-year duration of the simulation and N_{50} is the number of flowering plants extant at the end of the simulation. See Figure 1 for graphical definitions of the stated transition probabilities.

Model Conditions	λ	P(E)	N_{50} (±SD)
Baseline	1.135	0.0	18,000 (5,000 - 32,000)
$P_{11} = 0.25$	1.054	0.0	480 (82 - 890)
$P_{11} = 0.125$	1.019	0.0	100 (13 - 190)
$P_{11} = 0.0$	0.990	0.0	25 (2 - 49)
$G_{12} = 0.0042$	1.071	0.0	1000 (140 - 1900)
$G_{12} = 0.0028$	0.992	0.0	23 (1 - 52)
$F_5 = 20{,}475$	1.071	0.0	1000 (81 - 2000)
$F_5 = 13{,}650$	0.992	0.001	22 (4 - 40)
$G_{25} = 0$	0.978	0.001	14 (0 - 33)

It is important to remember during the evaluation of these results that the growth rates calculated in these initial models are based on a number of best-guess estimates for life-history parameters. As a result, the absolute value of, for instance, λ should not be taken as an exact predictor of

current population performance. Perhaps of greater value in this process is the analysis of changes in relative population performance resulting from perturbations made to individual life-history parameters such as seed set, seed germination rate, and seed bank characteristics as these are parameters for which only scant data are available.

Reducing the viability of seeds in a seed bank has a dramatic effect on the growth dynamics of the population. As the within-bank seed viability decreases from its baseline value of 0.50 to 0.0 (i.e., no seed bank is present), the deterministic growth rate falls to 0.990. Consequently, the characteristics of the seed bank may be an important factor in our understanding of the biology of this species.

In a similar fashion, both seed set and seed germination appear to play key roles in the population biology of the western prairie fringed orchid. Reducing either of these parameters by 50% yields a deterministic growth rate of 0.992. Stated another way, a reduction in seed germination rate from 0.6% to 0.3% generates a population that changes from deterministic growth to deterministic decline. Additional data on seed set, seed bank characteristics, and germination rate may therefore be highly valuable in increasing our understanding of the biology of this species.

If the direct transition from protocorm to flowering plant, as proposed by some orchid biologists, is removed from the life cycle of the species, the growth rate drops dramatically to $\lambda = 0.978$, equal to a 22% annual rate of population decline. The presence of this transition is very effective at generating large numbers of flowering plants, despite the small absolute value of the actual transition probability ($G_{25} = 0.0025$), and therefore provides the opportunity for greatly increased population growth. A more thorough knowledge of the nature of this transition is sorely needed.

Table 2. Elasticities calculated from the baseline population model for the Sheyenne National Grassland population of the western prairie fringed orchid.

	Seeds	Protocorm	Nonflowering	Dormant	Flowering
Seeds	0.1619				0.2053
Protocorm	0.2053				
Nonflowering		0.0829	0.0360	0.0094	0.0126
Dormant			0.0145	0.0025	0.0065
Flowering		0.1224	0.0904	0.0116	0.0389

The preceding discussion may be summarized by studying the elasticities of the baseline projection matrix. The elasticity of a projection matrix is basically a measure of the contribution of a particular element of the matrix to the magnitude of λ calculated from that matrix. Consequently, changes made to matrix elements with high elasticities result in proportionally larger changes in λ. As can be seen from Table 2, the projection elements with the highest elasticities are related to seed set, seed bank residency, and seed germination. Not surprisingly, the direct protocorm - flowering plant transition also shows a high elasticity. It is clear from this type of analysis that processes operating largely below ground make the most important contributions to population growth.

The simulated Sheyenne National Grassland population responds dramatically to changes in its life history that are characteristic of "good" and "bad" years for growth (Tables 3 and 4). In good years, λ increases by an average of about 10% under each scenario modeled while a decrease of about 10% on average is observed in bad years (compare λ in Tables 3 and 4 with Table 1). All models exhibit deterministic population growth in good years, while all but the original baseline model show deterministic population decline in bad years. Elasticities change in absolute value under these conditions, but the rank order among values changes very little. Overall, these observations are consistent with general expectations and point out the sensitivity of this and other plant species to significant swings in environmental quality over time in relatively harsh environments such as those observed in the Sheyenne National Grassland.

Table 3. Western prairie fringed orchid (*Platanthera praeclara*) population viability. Output for the deterministic and stochastic Sheyenne National Grassland models under "good year" conditions (see the text for a description of these conditions). The population growth rate per generation is given by λ, while P(E) is the probability of population extinction within the 50-year duration of the simulation and N_{50} is the number of flowering plants extant at the end of the simulation. See Figure 1 for graphical definitions of the stated transition probabilities.

Model Conditions	λ	P(E)	N_{50} (±SD)
Baseline	1.239	0.0	1.8×10^6 (410,000 - 3.1×10^6)
$P_{11} = 0.25$	1.169	0.0	100,000 (25,000 - 180,000)
$P_{11} = 0.125$	1.140	0.0	31,000 (6,400 - 56,000)
$P_{11} = 0.0$	1.113	0.0	10,000 (2,400 - 18,000)
$G_{12} = 0.0042$	1.175	0.0	120,000 (23,000 - 210,000)
$G_{12} = 0.0028$	1.096	0.0	3,600 (460 - 6,800)
$F_5 = 20{,}475$	1.175	0.0	120,000 (25,000 - 220,000)
$F_5 = 13{,}650$	1.096	0.0	3,800 (1,100 - 6,600)
$G_{25} = 0$	1.115	0.0	11,000 (1,700 - 20,000)

Table 4. Western prairie fringed orchid (*Platanthera praeclara*) population viability. Output for the deterministic and stochastic Sheyenne National Grassland models under "bad year" conditions (see the text for a description of these conditions). The population growth rate per generation is given by λ, while P(E) is the probability of population extinction within the 50-year duration of the simulation and N_{50} is the number of flowering plants extant at the end of the simulation. See Figure 1 for graphical definitions of the stated transition probabilities.

Model Conditions	λ	P(E)	N_{50} (±SD)
Baseline	1.043	0.00	220 (16 - 420)
$P_{11} = 0.25$	0.950	0.33	2.4 (0 - 5)
$P_{11} = 0.125$	0.911	0.95	0.4 (0 - 1)
$P_{11} = 0.0$	0.878	1.00	0
$G_{12} = 0.0042$	0.981	0.08	10 (0 - 22)
$G_{12} = 0.0028$	0.903	0.99	0
$F_5 = 20{,}475$	0.981	0.04	10 (1 - 19)
$F_5 = 13{,}650$	0.903	0.99	0
$G_{25} = 0$	0.837	1.00	0

Minnesota (Pembina Trail)

As a result of the broad differences in the stage-specific transitions defined in the western prairie fringed orchid model, this population displays consistently higher deterministic growth rates when compared to the corresponding Sheyenne National Grassland models (Table 5). The baseline model shows about 26% annual growth ($\lambda = 1.263$). As expected, λ decreases in the absence of a seed bank ($P_{11} = 0.0$), but not to the same extent as in the Sheyenne National Grassland model (compare Table 5 with Table 1). In fact, reducing the seed set and germination rate as well as eliminating the protocorm → flowering plant transition (G_{25}) all lead to smaller reductions in λ compared to the Sheyenne model.

As with the Sheyenne National Grassland model, the elasticity matrix (Table 6) indicates the large contribution made to the population growth rate by seed set and seed germination. However, the transition within the nonflowering stage (P_{33}) is considerably higher in the Minnesota model, presumably due to the larger P_{33} transition probability in the context of lower overall mortality in this environment.

Table 5. Western prairie fringed orchid (*Platanthera praeclara*) population viability. Output for the deterministic and stochastic Minnesota (Pembina Trail) models under baseline conditions (see the text for a description of these conditions). The population growth rate per generation is given by λ, while P(E) is the probability of population extinction within the 50-year duration of the simulation and N_{50} is the number of flowering plants extant at the end of the simulation. See Figure 1 for graphical definitions of the stated transition probabilities.

Model Conditions	λ	P(E)	N_{50} (±SD)
Baseline	1.263	0.0	5.2×10^6 ($2.3 \times 10^6 - 8.0 \times 10^6$)
$P_{11} = 0.25$	1.202	0.0	4.8×10^5 (1.9×10^5 - 7.7×10^5)
$P_{11} = 0.125$	1.177	0.0	1.8×10^5 (6.0×10^4 –3.0×10^5)
$P_{11} = 0.0$	1.155	0.0	72,000 (24,000 - 120,000)
$G_{12} = 0.0042$	1.205	0.0	5.5×10^5 (2.1×10^5- 8.0×10^5)
$G_{12} = 0.0028$	1.135	0.0	26,000 (8400 – 44,000)
$F_5 = 20{,}475$	1.205	0.0	5.3×10^5 (2.4×10^5-8.2×10^5)
$F_5 = 13{,}650$	1.135	0.0	27,000 (11,000 - 43,000)
$G_{25} = 0$	1.138	0.0	35,000 (14,000 - 56,000)

Table 6. Elasticities calculated from the baseline population model for the Minnesota population of the western prairie fringed orchid.

	Seeds	**Protocorm**	**Nonflowering**	**Dormant**	**Flowering**
Seeds	0.1116				0.1703
Protocorm	0.1703				
Nonflowering		0.0758	0.0940	0.0179	0.0203
Dormant			0.0197	0.0131	0.0057
Flowering		0.0946	0.0943	0.0074	0.1049

These simple deterministic models suggest that the different datasets collected in South Dakota and Minnesota do in fact represent biological differences among the populations, most likely due

to significant differences in the characteristics of the local environment experienced by each population. Perhaps the Sheyenne National Grassland population experiences higher levels of general environmental stress which limit the potential for enhanced population growth.

Stochastic Model Results

Simple analysis of deterministic, long-term growth rate does not take into account the variation in that rate which may occur because of variation in environmental conditions or the stochastic variation inherent to vital rates governed by binomial process, i.e., birth, death, sex determination, etc. When some or all of these sources of stochastic variation are added to deterministic models of population dynamics, a picture of risk emerges such that a population may have a non-zero risk of extinction despite a positive deterministic growth rate. This is particularly true in smaller populations or in those species with wide fluctuations in vital rates brought on by environmental fluctuations.

A series of stochastic models were developed for the western prairie fringed orchid that included annual variation in stage-specific transition probabilities resulting from environmental variation. The output from these models is presented here as the probability of flowering plant extinction (other stages may or may not be present) and the total number (actually, mean and standard deviation) of flowering plants present at the end of the 50-year simulation. As stated before, because of the number of guesses that were required in our model construction it is more appropriate to observe the differences in outcome between sets of models than to interpret specific outcomes as precise representations of reality.

Sheyenne National Grassland

The reduction and eventual elimination of a seed bank in the baseline Sheyenne National Grassland model produces a dramatic decline in the number of flowering plants predicted after 50 years (Table 1). If P_{11} is no greater than 0.125, the population is not expected to grow beyond its initial abundance of 100 flowering plants. These models show no risk of total extinction of the flowering plant stage, but in the absence of a seed bank there are only 25 flowering plants extant at that time with the risk of extinction perhaps becoming measurable beyond 50 years. In fact, some iterations in the $P_{11} = 0$ model declined very close to zero. Of even greater significance is the observation that when $P_{11} = 0.125$ and the corresponding $\lambda = 1.014$, the lower standard deviation in flowering plant abundance is just 13 individuals.

In similar fashion, a 50% reduction in the seed germination rate and the seed set led to a significant decline in the mean flowering plant abundance after 50 years. An even greater reduction in abundance is observed when the direct transition from protocorm to flowering plant is reduced to 0.0 (Table 1, bottom), where only 14 flowering plants remain after 50 years.

When average environmental conditions are favorable, flowering plants become very abundant under all conditions modeled (Table 3). However, under conditions indicative of a "bad year", the risk of flowering plant extinction (and, most likely, nearly total population extinction) becomes very high in all but the most favorable of scenarios and abundances drop to extremely low levels. Moreover, the predicted trajectory toward flowering plant extinction is quite rapid, i.e., on the order of 30-40 years.

Minnesota (Pembina Trail)
The Minnesota study population, based on expectations related to the very robust growth rates calculated from the life tables, shows extremely rapid growth and no risk of extinction throughout the duration of the simulations (Table 5). Once again, it is important to note that no carrying capacity is imposed on these populations so that unlimited population growth is possible under the appropriate conditions. While some of the final flowering plant abundances observed in these models may be clearly biologically unreasonable, it is instructive to take note of the potential for explosive growth of the populations should conditions become favorable.

Summary and Recommendations
A series of deterministic and stochastic models were developed for South Dakota and Minnesota populations of the western prairie fringed orchid (*Platanthera praeclara*) as a tool to investigate the current state of knowledge of the population biology of the species. Using a stage-based modeling approach, sensitivity analysis was used to assess the relative impact on population dynamics of changes to individual stage-specific transitions for which little field or laboratory data are available. In general, the bulk of the sensitivity these populations exhibit is manifest in those stages we know the least about: absolute viable seed set per flowering plant, the nature and extent of any seed bank, and the seed germination rate.

Comparison of the two study sites indicates that North Dakota's Sheyenne National Grassland population shows enhanced risk of population decline and perhaps even extinction under adverse environmental conditions. This is certainly not to say, however, that Minnesota's Pembina Trail population, which appears to currently display greater growth potential than its Sheyenne National Grassland counterpart, is immune from future risk.

Finally, it is vital to stress that these models are exploratory and preliminary. Despite this limitation of the process, however, a considerable amount of information has been obtained through comparative modeling and sensitivity analysis that has led to the development and enumeration of the following recommendations:

1) Seed stage: Seed bank study. We need to identify the residence time of seeds in the soil. This can be very important for population dynamics, particularly in a place like the Sheyenne National Grassland where suitable habitat shifts from the center to the perimeter of swales as conditions go from drought to flood. If seeds can persist in the soil in dry areas for several years before they become wetter, or if seeds can persist underwater until a drawdown, then this can allow the population to persist by a process of shifting recruitment across the landscape.

A possible approach would employ seed packets, as currently in use by Hull-Sieg, to see how many seeds germinate and become protocorms and seedlings after one year in the ground, 2 years, etc.

2) Protocorm: **Protocorm study.** In these models we have included a direct transition from the protocorm stage and the flowering stage. This would indicate that a protocorm could become a flowering plant, bypassing a vegetative, aboveground stage. The model appears to be very sensitive to the presence of this transition. With the transition the population grows; without it the population declines. This transition was included because both the Sheyenne and Minnesota

datasets showed cases of plants appearing for the first time as flowering plants (18% of new appearances for Sheyenne and 10% of new appearances for Minnesota). There are 2 possible explanations for this: 1) these plants were present as protocorms prior to appearance, 2) these plants were present as dormant plants prior to appearance. Explanation 1 was chosen because explanation 2 appeared to have a problem, namely, that some flowering plants appeared more than 3 years after the study began. It was thought to be unlikely that these plants could have remained dormant for this long. However, in the Minnesota study, 12% of dormant plants returned after more than 3 years so perhaps explanation 2 should not be ruled out. Explanation 1 has its own problems. In vitro culture and the literature suggests that plants remain in the protocorm stage for several years and have not achieved a size sufficient for flowering. Also, data from Pleasants indicates that plants must achieve a threshold size as vegetative plants before they can flower. More work needs to be done on this stage.

The seed packets could again be used as a possible approach. Protocorms that appear in the packets can be removed and placed in new packets and followed over the years to determine their transition time to other states. To reduce damage to protocorms while removing them from packets, seed packets can be removed in the fall when plants have stopped growing. It should be noted, however, that mortality from exposure, damage, etc. could bias examination of treatment effects (Hull-Sieg, pers. comm.).

3) Seed set: Obtain estimates of number of seeds per mature capsule and determine whether "not so plump" capsules have any seeds (or viable seeds). Also determine variability in seed capsule production. The seed output has a strong effect on population growth in the model. We need a better handle on seed production.

4) Dormancy: Begin plot studies of marked plants at several sites, especially upland prairie sites. It will be necessary to follow plots in which all plants present are marked. Dormancy can be inferred when marked plants disappear for a period of years and then reappear. The proportion of plants which disappear then later reappear needs to be determined as well as the length of the dormancy period.

5) Recruitment: Begin plot studies of marked plants at several sites, especially upland prairie sites. It will be necessary to carefully examine plots every year to determine if new plants have appeared. The appearance of plants can be related to environmental variables such as precipitation.

6) Application of Models to Other Areas and Habitat Types. Our demographic models to date are primarily for wet meadow, large population, sites at the northern end of the species range. We need demographic data for upland prairie sites and populations in other parts of the species range. With regards to populations in other parts of the species range such as Nebraska, data from small sites, upland prairie populations and wet meadow populations in the sandhills would be most helpful with regards to furthering our understanding of this species.

7) Comparison of Sheyenne and Minnesota data sets: It will be useful to take the raw data from both data sets and recast transition probabilities in the same way for both so they can be compared. Determine the impact of environmental variables on transition probabilities. Data

collection efforts in these areas should be continued. The Sheyenne Model was based on a four-year data set; this in particular should be expanded to include more years. The impact of environmental variables and management treatment, and perturbations on transition probabilities and the sizes of different life stages also need further examination and study.

8) Model explorations: Determine if the model can accommodate autocorrelations across years and across transition probabilities within years. Often wet years are followed by wet years and dry by dry. The cumulative effect of repeated conditions can be significant. Also, in the stochastic model some transition probabilities are affected similarly, i.e. if it is a bad year for flowering plants it should also be a bad year for vegetative plants.

Working Group members: Alexis Duxbury, Carolyn Hull-Sieg, John Pleasants, Todd Suess, Brian Winter, Kevin Church, Phil Miller.

POPULATION AND HABITAT VIABILITY ASSESSMENT (PHVA) FOR THE WESTERN PRAIRIE FRINGED ORCHID (*Platanthera praeclara*)

Eugene Mahoney State Park
Ashland, Nebraska
27-30 April 1997

Final Report

Section 5
Ex-situ Working Group Report

EX-SITU CONSERVATION REPORT AND RECOMMENDATIONS

Introduction

Nebraska's known populations of Western Prairie Fringed Orchid are fragmented and limited in number, therefore it is imperative that a pro-active approach to ex-situ conservation be initiated. The benefits to be derived from this approach are numerous. Initially the experience with in-vitro propagation, conventional propagation, and field methods would provide a backstop to prevent and possibly reverse extinction. It can also provide a means of species reintroduction to areas that previously supported this plant. With a base population of reintroduced plants further studies to explore the plants biology and life history can be initiated without affecting natural populations. Experience and knowledge gained with the Western Prairie Fringed Orchid can provide techniques that may be applicable to similar species. Thus, the need for ex-situ conservation methods and techniques can be invaluable to the continued existence of this species and to expanding our knowledge of general orchid biology.

Working Group Goals:

1. To prevent extinction of the Western Prairie Fringed Orchid (*Platanthera praeclara*). Steps must be taken to provide alternative means of propagation and germplasm storage as a means of preserving the genetic diversity present in *in-situ* populations.

2. To protect and conserve the Western Prairie Fringed Orchid *in-situ*, education and public awareness is imperative.

3. To apply research tools *ex-situ* is necessary in order to understand the biology of the species and basic cultural requirements in *in-situ* populations.

Issues and ideas surrounding each goal:

1. Conservation: Conservation goals include establishment of effective germination protocols for the species. This encompasses all protocols currently under development including *in-vitro* (From) and *in-situ* (Hull-Sieg) techniques. This plant species has not previously been successfully propagated using traditional propagation methods and much more research needs to be done in this area. Studies need to cover the plant's propagation from seed all the way through the growing-on of mature specimens in order to elucidate the plants entire life cycle. At this time, juvenile plants in the form of protocorms have been produced *in-vitro* by From and *in-situ* by Hull-Sieg. Further growth and acclimatization strategies are necessary to produce plants capable of survival under natural environmental conditions. There is no published literature currently available which specifically addresses the issue of cultural requirements for growing-on of *Platanthera praeclara* plants. This issue is projected to be investigated over the next year or two (Hull-Sieg, Johnson, From).

Links between researchers working on the species need to be established, including the mycorrhizal associations(s) identified (Zelmer, Antlfinger) which impact on in-vitro and field studies. Communication between researchers would be facilitated through uses of databases such as the one at the Center for Plant Conservation's (C.P.C.) and computer networks such as Listserv or World Wide Web.

The field population survey planned for Nebraska in 1997, by the Nebraska Game and Parks Commission, is critical for all further study within the state. Monitoring of populations can be modeled on work currently being done in Minnesota and North Dakota (Sather, Hull-Sieg). This includes wild populations, *in-situ* germination studies, and/or any field trials conducted with plants propagated by human intervention.

Cryopreservation techniques for plant materials will be investigated through communication with botanical gardens conducting research on native terrestrial temperate-zone orchids.

All related field studies on *P. praeclara*, such as seed germination, plant viability, longevity, developmental biology, and pollination strategies (natural vectors, as well as hand-pollination studies), are extremely important for conservation of the species. Soil analyses and requirements region-wide have direct applicability to *ex-situ* propagation, in addition to their obvious value for greater understanding of the wild populations present in Nebraska.

Genetic analysis is being conducted at the Center for Conservation and Research at the Henry Doorly Zoo (Hancock/Louis/O'Kane) for *P. praeclara* wild populations, as well as the closely related species, *Platanthera leucophaea* (the Eastern Prairie Fringed Orchid). These analyses will establish the parameters of genetic variations for each of the two species, as well as define more clearly the taxonomic differences that are believed to exist between the two species.

Reintroduction strategies are considered a valid option for the future of the species within the region, to be conducted by specifically drawing on details of the translocation project completed in Manitoba, Canada (Johnson). Reintroduction field trials, such as that conducted on another *Platanthera* species in Ontario, Canada, (Anderson) would provide opportunities for valuable information leading to the overall goal of preventing this plant's extinction in the region. Any reintroduction trials on selected sites must be pre-approved by the appropriate governmental agencies.

2. Education: The general public needs to be made aware of the intrinsic value and worth of the Western Prairie Fringed Orchid as part of Nebraska's natural heritage. Through *ex-situ* conservation both public and professional education can be assimilated and disseminated. Currently the Nebraska Statewide Arboretum and the Henry Doorly Zoo are collaborating on curricula for sixth grade level information on rare plant species of the Great Plains. The Nebraska Game and Parks Commission has developed a brochure describing the Western Prairie Fringed Orchid which can be utilized by schools, garden centers, nurseries and garden clubs. The Center for Plant Conservation (CPC) has a site on the World Wide Web (WWW) pertaining to graduate programs and research programs concerning the Western Prairie Fringed Orchid. In order to enhance current educational activities, we propose the development of web site materials to reach a wider audience. Development of a campaign for outreach at state and county fairs or Earth Day activities could also prove beneficial for public awareness and education. Development and distribution of a guide to Nebraska's native species and habitats for distribution and in other roadside locations would also increase public awareness. Potential harvesting problems could be addressed by offering alternatives to Western Prairie Fringed Orchid wild collecting such as *Bletilla,* a temperate orchid. In order to prevent poaching of the species, it may be necessary to develop techniques of mass production for commercialization, if

at some point that should be deemed necessary. The use of volunteers from organizations such as the Henry Doorly Zoo, native plant societies and garden clubs should disseminate information, perhaps through an educational slide program, and to assist with conservation programs or studies. These actions should be encouraged in order to further develop a general public awareness of the orchid and other rare species in Nebraska. This educational effort will foster a sense of ownership and responsibility for endangered species by the citizens of Nebraska and increase support for Nebraska Game and Parks commitment to protecting Nebraska wildlife.

Recommendations:

A significant proportion of the following recommendations can be realized, often as extensions of existing programs, through the initiation by Nebraska Game and Parks Commission of multidisciplinary collaborative efforts with the following institutions, acting in support of NGPCs effort to preserve and protect this species: the Center for Plant Conservation, the Henry Doorly Zoo, the University of Nebraska at Lincoln, the USDA Plant Materials Center, and the Nebraska State Arboretum.

1. Develop effective means of plant propagation including protocols for germination and growth to maturity, with the goal of increasing the total number of plants and maintaining genetic diversity.

2. Develop protocols for short and long term germplasm storage, such as seeds, callus and protocorms.

3. Utilize *ex-situ* germination and culture studies to document developmental biology such as plant anatomy and physiology and to provide additional material for further research studies of genetics, morphology and longevity.

4. Undertake field studies of *in-situ* populations to compare developmental responses to *ex-situ* studies and to provide information such as soil requirements or mycorrhizal associations which may facilitate *ex-situ* cultivation.

5. Develop reintroduction techniques through controlled field research utilizing both direct and *in-vitro* transplants as well as direct seeding experiments.

6. Develop public display gardens, without disrupting wild populations, for public education. To reach a broad audience gardens could be developed at Nebraska Statewide Arboretums, zoos and state or federal parks.

7. Produce and distribute printed materials such as brochures, posters and interpretive signs for public education.
8. Develop education materials for use in school curricula introducing Great Plains rare, threatened and endangered species including the Western Prairie Fringed Orchid.

9. Recruit local nursery and garden centers and involve cooperative extension programs as an educational outlet to reach the general public.

Commitments for Implementation by Henry Doorly Zoo/ Terri Gouveia

1. By July 31,1997 - Meet with Nebraska Statewide Arboretum and Nebraska Game and Parks to see if interpretive materials that are being developed could be designed to include NGP. (Recommendation # 7) *Completed*

2. Initiate conversation with Kew Gardens, London for protocol on beginning experiments with cryopreservation at HDZ on Western Prairie Fringed Orchid seed. (Recommendation #2)

3. Contact Minnesota for a possible seed source since they hold the largest population (Brian Winter). (Recommendation #2) *In progress*

4. By December 1997 - Follow up with Anukrtiti Sud, Center for Plant Conservation on WWW potential as well as collaborative educational materials. (Recommendation #8)

5. Continue to support Marge From's research laboratory and micro propagation unit at HDZ (in affiliation with the Department of Horticulture, University of Nebraska – Lincoln) as well as field work at Valentine. (Recommendation #1)

6. By July 1997 - Meet with Nebraska Game and Parks to look at joint opportunities for educational aspects between Mahoney State Park and Henry Doorly Zoo's new facility "Nebraska's Safari Park." (Recommendation #7) *Completed*

7. Initiate conversation with NGP on possible assistance of organizing the various volunteer forces for survey and conservation statewide. (Recommendation #4) *Completed*

Working Group Members: Terry Conway, Paul Currier, Margaret From, Terri Gouveia, Facilitator, Kent Pffiefer, Paul Read, Anukriti Sud, Erika Szendrak, Richard Wynia

POPULATION AND HABITAT VIABILITY ASSESSMENT (PHVA) FOR THE WESTERN PRAIRIE FRINGED ORCHID (*Platanthera praeclara*)

Eugene Mahoney State Park
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Final Report

Section 6
Management Working Group Report

MANAGEMENT REPORT AND RECOMMENDATIONS

The goal of all management actions should be the maintenance and enhancement of the western prairie fringed orchid and its associated native prairie habitat. Management should include not only the implementation of management practices but also the use of conservation measures to insure the long-term implementation of the practices.

Basic to the implementation of an optimal management plan is understanding of the effects of various management practices and their variables on the western prairie fringed orchid. The number of practices, the combination of their use, multiplied by practice variables, climatic conditions, and hydrologic factors results in an almost infinite complexity of management practice effects. This illustrates the need to conduct research on the various management practices and their variables in order to determine their effects and subsequently identify the best management practices. These practices can then be used to devise an optimal management plan for each population.

While the recovery plan provides a very good outline of management issues and research areas, it is evident from the recovery plan and this PHVA workshop that there is a considerable lack of verifiable data on the effects of various management practices on the species. This emphasizes the need for research to determine these effects in order to be able to implement optimal management and successfully address the variety of sites and conditions in which the species occurs throughout its range. It should also be recognized that the research recommendations in the life history section were made with the goal of ultimately influencing management of the species. Therefore, there should a concerted effort to integrate research on life stages and management practices when appropriate.

Management research should focus on those practices known to be important for the prairie community in general. Key to understanding these practices is the need to understand the variables associated with each practice. Therefore, research conducted for each of the practices should also include the variables associated with it.

Seasonal timing should address the potential for variations in practice effects based on the plants growth stage. Recognizing that growth stages integrate, four stages have been identified as important for evaluating management effects. They are: 1) early season growth (plant emergence to two-leaf stage), 2) maturation growth and flowering (stem formation and flowering), 3) seed maturation and dispersal, and 4) post-seed dispersal. The identification of the stages is broadly defined based on general plant phenology. The plant does not necessarily progress through these stages within one growing season. In fact, as much as 75% of the plants are vegetative in a given growing season, and this includes plants that were flowering in the previous year (Hull-Sieg, pers. comm.). Also crucial to evaluating management effects is the evaluation of influences of climatic effects. Climatic variables to be considered include; precipitation amount, timing of precipitation, annual precipitation patterns, temperature, and evaporation rates.

Research should address the effects of variables related to hydrologic factors on management practices and address sources of changes in hydrology. Hydrologic factors related to orchid habitat are different for wet meadow habitat as compared to processes influencing habitat on

upland sites and vary geographically. For wet meadow habitat, specific areas of research should examine the effects of: a) above normal annual precipitation, b) drought, c) ditching, d) reduction in instream flow, and e) well placement. For upland habitat, specific areas of research should examine the effects of: a) above normal annual precipitation, b) drought, c) reduction in tract size, d) increased evaporation due to conversion of surrounding land for crop production, urban development, etc., and e) decreased infiltration and soil water retention due to conversion of surrounding land for crop production, urban development, etc.

In devising a management plan for a population it should be understood that a plan is not intended to be static, and that it should change both as the conditions of the site change and as knowledge of the effects of management practices increases. For this reason a management plan should encompass both a short-term and long-term management approach. Although we may not know the direct effect of certain practices on the orchid there is a considerable background of knowledge and understanding of such practices as applied to the prairie ecosystem, of which the orchid is a component. Therefore, for the short-term a management plan should utilize those tools and follow a prescription that is known to maintain and enhance the prairie community. As new understanding is gained on the effects of specific management practices, those beneficial to the species can be incorporated into the plan.

When available, documented, annual land use, histories should be assessed. This information can be used both to help structure and direct management research and to devise short-term management plans. In conducting research and devising management plans it should be recognized that applying certain methods and practices to small, isolated populations may produce highly detrimental effects or make the population vulnerable to catastrophic events that a would not be the case for large populations.

In determining the optimal conservation strategy for a given population it is necessary to consider factors that will influence and may limit the type and level of conservation and management practices that can be implemented. Foremost is land ownership and land use priority.

Basic land ownership can be categorized as:

1. Public lands
    a. Federal
    b. State
    c. Local
2. Private
    a. Individual
    b. Non-governmental organization (NGO)

Land use priority can vary from being essential for hay production for cattle to game species production to being dedicated to prairie community conservation. Under the combination of land ownership and land use priority it may be possible to initiate more effective management practices and a higher level of conservation protection on a tract of private land dedicated to prairie conservation than on public land. In any instance, an understanding of the needs and objectives of all involved parties is crucial to being able to develop a conservation plan

appropriate for a given orchid population. For instance, if a site is utilized for hay production an agreement may be devised where, in exchange for technical support in identifying the presence of and marking plants, the landowner will mow around plants, mow seasonally to avoid impact, or mow on a rotational schedule. While this may not be an optimal plan it is a significant level of protection over no agreement.

Recommendations

1. Determine the effects of hydrologic factors on management practices and address sources of hydrologic modifications including:
    - A) Impacts of changes in groundwater levels on wet meadow habitat.
        - a) Higher than normal precipitation
        - b) Drought
        - c) Ditching
        - d) Reduction in instream flow
        - e) Well placement
    - B) Impacts of changes in hydrologic processes on upland habitat.
        - a) Higher than normal precipitation
        - b) Drought
        - c) Reduction in tract size.
        - d) Increased evaporation due to conversion of surrounding land use for crop production, urban development, etc..
        - e) Decreased infiltration and soil water retention due to conversion of surrounding land use for crop production, urban development, etc..

2.Conduct research on current prairie ecosystem management practices and variables to determine their effects specific to the western prairie fringed orchid. These practices and associated variables include:

- A. prescribed burning; seasonal timing, frequency, rest/rotation
- B. grazing; seasonal timing, frequency, duration, stocking rate, rest/rotation
- C. haying; seasonal timing, frequency, rest/rotation
- D. noxious weed control;
    - a) specific application of management practices 1.1), 1.2), & 1.3).
    - b) mechanical - seasonal timing, frequency
    - c) biocontrol - seasonal timing, frequency, impact on other native species
    - d) chemical- seasonal timing, frequency, amount
- E. pesticide use;
    - a) direct and indirect impact to plant - seasonal timing, frequency, amount
    - b) impact to prairie community - seasonal timing, frequency, amount
    - c) impact to pollinators - seasonal timing, frequency, amount
- F. fungicide use.

3. Determine effects of management practices on seasonal growth stages of the orchid. The identified stages are:

- A. early season growth (plant emergence to two leaf stage)
- B. maturation growth and flowering (stem formation and flowering)
- C. seed maturation and dispersal

D. post-seed dispersal

4. Determine effects of climatic conditions in conjunction with management practices. Climatic factors to be evaluated include:

A. precipitation amount
B. timing of precipitation
C. annual precipitation patterns
D. temperature
E. evaporation rates

5. Integrate management and life history research when appropriate.

6. Develop and implement management plans using a short-term and long-term approach.

A. Use management tools known to maintain and enhance the prairie community to implement short-term plans.
B. Under a long-term mange approach incorporate research results into plans to achieve optimal management practices.

7. Use documented annual land use histories to structure and direct management research and devise short-term management.

8. Identify and address factors that may influence and limit the type and level of conservation and management practices that can be implemented. These include:

A. Landownership categories.
    a) Public lands
        i. Federal
        i. State
        ii. Local
    b) Private
        i. Individual
        ii. Non-governmental organization (NGO)
B. Land use priority
    a) Forage production
    b) Prairie/orchid conservation

Working Group Members: Paul Currier, Michael Fritz, facilitator, Tim Knott, Len McDaniel, Kent Pffiefer, F. M. Reece.

POPULATION AND HABITAT VIABILITY ASSESSMENT (PHVA) FOR THE WESTERN PRAIRIE FRINGED ORCHID (*Platanthera praeclara*)

Eugene Mahoney State Park
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Final Report

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POPULATION AND HABITAT VIABILITY ASSESSMENT (PHVA) FOR THE WESTERN PRAIRIE FRINGED ORCHID (*Platanthera praeclara*)

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Appendix I.
Workshop Presentations

Demography of Two Populations of the Western Prairie Fringed Orchid

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Two populations of the Western Prairie Fringed Orchid were monitored. One, at Sheeder Prairie in south central Iowa, is representative of many of the populations in the southern part of the species' range, which occupy fairly dry prairies. The other, at Pembina Trail Scientific and Natural Area in north western Minnesota, is representative of many of the populations in the northern part of the
species' range, which occupy mesic habitats. Monitoring began in 1991 (Sheeder) and 1992 (Pembina) with a set of marked flowering plants. Each year thereafter these plants and a new set of marked flowering plants were monitored. The status of each plant (absent, vegetative or flowering) was assessed and plant size (leaf area) was measured. The number of flowers on flowering plants and fruit set was also recorded.

The questions addressed in this study were:
1) What is the annual mortality (permanent disappearance) rate for these plants?
2) What are the transition probabilities from one status to another between years?
3) What is the fruit set of plants?
For the above questions I was also interested in the variation in the values of these parameters among years and between sites.
4) What is the effect of spring burns?
This question was examined only at Sheeder Prairie.

1) The 2 sites did not differ much in annual mortality. The average mortality rate was 15.6% for Sheeder and 17.8% for Pembina. There was quite a bit of variation in these rates among years, ranging from 9-27% at Sheeder and 6-33% at Pembina.

2) Transition probabilities were only analyzed for Pembina. Flowering plants that survived to the next year flowered again with a probability from 0.20 to 0.34 over 3 years but with a 0.81 probability in one year. The majority of plants that returned the next year as vegetative plants had 2 leaves. The size (leaf area) of a plant in one year was positively correlated with its size the previous year. Vegetative plants that were larger in a particular year were more likely to flower the next year.

3) The average fruit set at Sheeder was 23%, at Pembina 21% and at a third site (Sheyenne National Grassland) 18%. Over all sites and years fruit set ranged from 6-33%. Fruit set values are for plants that completed flowering. In some years plants did not complete flowering because of herbivory (in 2 of the years at Pembina 36% and 74% of inflorescences were removed by deer) or inflorescence abortion (in 1 year at Sheeder after a dry spring 100% of inflorescences aborted).

4) The response to spring burns (in April) at Sheeder was dependent on the amount of precipitation, particularly during the months of May and June. Following a burn, if May and June were drier than normal, plants on the burned plots did more poorly than those on unburned areas.

If these months were wetter than normal, plants on burned plots did better. May and June precipitation levels the following year produced a similar differential effect.

One conclusion that can be drawn from these data is that the 2 sites are surprisingly similar in demography, despite major habitat differences. The one exception is that there is a higher probability of inflorescence abortion at Sheeder. It is also encouraging that pollination is occurring on a small site such as Sheeder Prairie which does not have the long-tongued moth species which are the coadapted pollinators. In fact pollination levels are similar to those of metapopulations, such as Pembina, which have the appropriate pollinators. Another important point is that precipitation levels during the growing season play a key role in determining the well being of plants in a particular year and their status the following year.

Research Update on the Western Prairie Fringed Orchid on the Sheyenne National Grassland, North Dakota[1]

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The western prairie fringed orchid (*Platanthera praeclara*) is a showy plant that was once widespread throughout much of the tallgrass prairie in the central United States and southern Canada. However, populations of the orchid are now mostly small, scattered and isolated. Populations of the western prairie fringed orchid are restricted to the Great Plains states and Manitoba; the three largest metapopulations occur in Minnesota and North Dakota in the United States and in southern Manitoba in Canada. The metapopulation in North Dakota is on the Sheyenne National Grassland which is managed by the Custer National Forest.

Due to concerns about the limited number of populations and their relative isolation, the orchid was federally listed as a threatened species in 1989. The demise of this species parallels the loss of over 99% of the tallgrass prairie in the central United States and Canada due to the conversion of prairie to crop land and the encroachment of other human development. As a result, this ecosystem is one of the most threatened in the United States. Therefore, our research on the western prairie fringed orchid focuses not only on understanding its life history and habitat needs, but also on strategies for restoring the tallgrass prairie ecosystem and its associated wetlands.

The western prairie fringed orchid has many attributes that make it difficult to study, and many questions remain about its life history. It is a perennial plant that has two distinct life states. In some years, a given plant will remain vegetative throughout the growing season, and in other years it will flower. The vegetative form consists of one to four basal leaves up to 6 inches [15 cm] long. Flowering plants are taller (up to 75 cm in height), with as many as 20 or more cream-colored flowers arranged on a spike. The flowers produce a fragrance at night that attracts hawkmoths, which pollinate the orchid. In years when extensive flowering occurs, and pollination is successful, thousands of dust-like seeds are produced.

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[1]Synopsis of papers previously published:

Sieg, C.H. and R.M. King. 1995. Influence of environmental factors and preliminary demographic analyses of a threatened orchid, *Platanthera praeclara*. American Midland Naturalist 134:307-323.

Sieg, C.H. and A.J. Bjugstad. 1994. Five years of following the western prairie fringed orchid (*Platanthera praeclara*) on the Sheyenne National Grassland, North Dakota. Pages 141-146 In Wickett, R.G., P.D. Lewis, A.Woodliffe, and P. Pratt (Eds.). Proceedings of the Thirteenth North American Prairie Conference. Preney Print and Litho Inc., Windsor, Ontario, Canada.

Richardson, V.J.N., C. H. Sieg, and G.E. Larson. 1997. In situ germination of the western prairie fringed orchid (*Platanthera praeclara).* [abstract]. Abstracts of the 50th Annual Meeting of the Society for Range Management 50:56.

Wolken, P.M. 1995. Habitat and life history of the western prairie fringed orchid (Platanthera praeclara). M.S. Thesis. Dept. Plant, Soil and Insect Sciences., University of Wyoming. 93 pp.

At the end of the growing season, plants produce a new perennating bud and tuber which develop into the new root system and shoot for the following growing season. In this manner, populations may persist for some time. However, seed establishment is required for recruitment of new individuals. Almost nothing is known about its germination ecology, and until recently efforts to propagate plants in the laboratory have been unsuccessful. Anecdotal observations have suggested that up to five years may be required for plants to emerge aboveground following germination. By using an innovative technique whereby the dust-like seeds are placed in a fine mesh packet and placed in the soil, we now know that seeds may germinate the first growing season following planting, and based on the presence of seedlings in some of our samples collected late in the first growing season, it is possible that new individuals appear aboveground as soon as the second growing season following seeding. It is very likely that mycorrhizae is required for germination and/or other developmental stages, and the absence of this beneficial fungi makes propagation unlikely.

Until recently, the orchid was thought to be long-lived, and to have periods of dormancy when tubers subsisted underground for a growing season or more. Long-term data, involving monitoring permanently marked plants over 10 years or more are needed to answer these questions of longevity and dormancy. Therefore, in collaboration with the Custer National Forest, in 1987, we established permanent transects in areas of orchid concentrations and marked a total of 160 individual plants. By 1994, only 4% of the originally marked orchids were observed aboveground. Marked plants lived up to eight years, but the majority of the plants we monitored were present only one or two growing seasons. Further, once an orchid disappeared, it rarely reappeared. The question now is whether droughty conditions in the first four of seven years of our monitoring, followed by flooding in 1993, may have reduced survival and/or increased dormancy rates beyond levels that could be expected under optimum periods.

Data collected from these transects plus additional research plots have also provided the foundation for examining other questions such as the role of soil moisture and the effect of management activities on maintaining populations of the western prairie fringed orchid. The most significant factor influencing orchid numbers is soil moisture. The western prairie fringed orchid usually occurs in wetland swales. Wetland soils supporting the orchid on the Sheyenne National Grassland are usually Mollisols, but also include Entisols and Inceptisols. During the drought in the early 1990's, orchid numbers dwindled and could only be found in the deepest swales. Flooding since 1993 has enhanced orchid numbers, but new orchids are appearing on higher landscape positions.

The transects we established on the Sheyenne National Grassland in 1987 encompassed a variety of management regimes, including grazing, burning, a combination of grazing and burning, burning without grazing, and neither burning nor grazing. The number of orchids has varied dramatically on individual swales and from year to year. However, we have not been able to detect any consistent effects of these management regimes on orchid populations. Other researchers have suggested that seasonal mowing, grazing and burning on the Sheyenne National Grassland may enhance colonization by the orchid, in response to the reduced competition from other species. However, our data do not support this theory. We are continuing to monitor plots that were burned in the fall of 1990, and have recently set up plots that will allow us to gain a better understanding of the influence disturbances such as fire and grazing have on orchid populations.

Our research on the Sheyenne National Grassland has also helped us to characterize the plant community that supports orchids, and to develop a model that managers can use to identify suitable habitat for reintroducing orchids in this area. Vegetation and edaphic parameters of swales that support orchids were compared with adjacent swales devoid of orchids to identify factors that limit the presence of orchid populations. High soil moisture content was a critical requirement for orchid habitat. A logistic regression model correctly classified 87% of the swales as either orchid-supporting or non-orchid-supporting using five variables: percent canopy cover of baltic rush *(Juncus balticus)*, percent canopy cover of hedge-nettle *(Stachys palustris)*, soluble oil magnesium, August surface soil moisture, and canopy cover of sedges *(Carex* spp.). This model would be most beneficial if used to identify additional habitat when attempting to expand a population or re-establish one, providing that transplanting or germination success is likely.

In characterizing the plant community of these swales, it has become apparent that invasive species threaten the quality of orchid habitat on the Sheyenne National Grassland. Kentucky bluegrass *(Poa pratensis)* has become a conspicuous component of the vegetative community in swales where the orchid occurs. Kentucky bluegrass tends to dominate and out-compete other plants, and orchid density in some years on the Sheyenne National Grassland was negatively correlated with Kentucky bluegrass cover. The invasion of leafy spurge (*Euphorbia esula*), a perennial noxious weed, is a serious threat to wetland habitats on the Sheyenne National Grassland. Unless effective methods are developed to control the spread of leafy spurge, orchid habitat on the Sheyenne National Grassland remains at risk of being taken over by this invasive species. Current efforts to curb leafy spurge include herbicides, biological control, and herbivores such as goats and sheep. We are collaborating with universities and other agencies to overcome the severe obstacles of controlling leafy spurge without severely impacting not only the orchid, but other components in this already imperiled ecosystem.

Platanthera praeclara; A Threatened Temperate Prairie Orchid Species

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Abstract

Micropropagation experiments and seed germination studies for Platanthera praeclara are discussed, based on research conducted in 1995 and 1996. Seeds were used for the initial stages, in order to preserve the most genetic diversity. Permits allowed limited seed collection and seed capsules were collected in the autumn of 1995, and 1996. Seeds were aseptically cultured on agar-gelled media to test for germination response within glass culture vessels. Germination took place on two of the media tested several months after initiation of the cultures. Permission was also obtained to conduct a limited hand-pollination study on two Western Prairie Fringed Orchid populations. It must be borne in mind that interpretations of this study are limited by the small sampling size of plants allowed for the hand-pollination study. Further scientific research has been authorized by state and federal agencies. Continued investigation will allow greater understanding of the value of micropropagation as a means to assist in preservation of the species and the production of plants for possible reintroduction efforts.

Key words: Platanthera praeclara, Western Prairie Fringed Orchid, WPFO, *in-vitro*, micropropagation.

Introduction

The Western Prairie Fringed Orchid, (Platanthera praeclara), is listed as a Threatened Species and is afforded protection under federal and state Endangered Species acts. Federal and state permits were obtained for the study. In the past, according to the Nebraska Game and Parks Commission (Fritz, 1993), this species has resisted attempts at propagation using traditional techniques. This study is investigating asymbiotic micropropagation as a means to produce WPFO plants for it's conservation, future reintroduction to selected sites within the plant's historic range, and for educational displays.

Comprehensive, integrated conservation strategies combining micropropagation techniques and species management *in-situ* are being employed for the conservation of the Great Plains native terrestrial orchids. Although the primary focus is on *Platanthera praeclara*, parallel experiments are being conducted on other Great Plains orchids. The techniques developed for *P. praeclara* have also demonstrated applicability to germination for some of the other native orchids.

Several issues are being addressed in this ongoing study: 1) methods of germinating *P. praeclara* seeds *in-vitro*, 2) the study of seed structures and their effects on germination and 3) a limited hand-pollination study to assess the possibility of inducing greater fruit-set in a wild population.

M. From, 1997

Germination response

Mature seeds collected in late summer of 1995 and 1996 were surface disinfested in an 8% calcium hypochlorite/92% distilled water solution, or a 10% sodium hypochlorite/90% water solution for varying lengths of time, and cultured aseptically on several agar-gelled media. Seeds were sown on a modified FAST medium (Fast, 1982) with modifications by (Anderson, 1990) and (From, unpublished), a one quarter strength Vacin and Went (1949) and a new medium designated as P/C (From, unpublished). Seed culture vessels were all placed in continual darkness at 23°C ± 2, alternating with cold stratification at 5°C, for varying lengths of time: 4 weeks, 8 weeks and 12 weeks.

Germination of 1995 seeds began after five months in culture. Resulting protocorms were recultured on fresh media when they possessed a small shoot initial and the entire protocorm measured 5 mm in length, or longer.

Seed Structures

Mature seeds were photographed in the dry state, as well as after soaking in a 10% bleach solution for various lengths of time. Photographs were made using a Nikon FX-35 DX camera mounted on a Nikon Labophot-2. This was done to examine microscopically what effects disinfestation techniques have on the orchid seeds' hard seedcoats when aseptic cultures are initiated.

Hand Pollination Study

Permits were obtained to conduct limited hand pollination on one privately owned upland prairie, site #1, and on a low-lying prairie swale population located on federal lands, site #2. This study was first conducted in 1996. Its primary purpose was to assess whether there is any merit in using human assisted pollination to improve seed set for the orchid genotype(s) in wild populations located in the far western reaches of the orchid's range, where it's populations are small, under threat of human encroachment and the orchid population numbers are believed to be in decline.

Materials and Methods

Germination Response

Seeds collected in late summer of the years 1995 and 1996, from sites 1 and 2, were surface disinfested and cultured aseptically in culture vessels containing several different agar-gelled media. Seeds were disinfested in a 8% calcium hypochlorite solution or a 10% sodium hypochlorite solution. Cultures were all initiated with dry, mature seeds.

M. From, 1997

Seeds were sown on a modified FAST medium (Fast, 1982) with modifications (Anderson, 1990 and From, unpublished), a modified VW medium (Vacin and Went, 1949), and a new medium designated as P/C (From, unpublished). Individual culture vessels were given alternating cold treatment at 5°C, and room temperature 23-25°C regimes, all in continual darkness. Cold treatments were applied for 4, 8, and 12 weeks. Seed germination of temperate zone terrestrial orchids is slow and seeds within a single culture vessel can continue to germinate over 12 months or longer, (Stoutamire, 1974).

Seed Structures

Understanding the physical characteristics of *P. praeclara* seeds is a key element to successful germination of the species. The seedcoat is not readily permeable by water, and this exerts profound impact on the germination rate.

Dry, mature seeds were photographed at 200x and 400x. Dry seeds displayed a dark, opaque seedcoat. Wet seeds were photographed after soaking in a sodium hypochlorite solution for varying lengths of time.

Two orchid sites were chosen to conduct human-assisted pollination. One site was an upland site on a privately-owned prairie and the second site was a wet, low-lying prairie swale. The sites are located approximately 350 miles apart.

Twenty-eight plants were randomly chosen for study at site #1 and 10 plants were randomly chosen at site #2. Each individual inflorescence displayed a minimum of 5 fully-expanded, intact flowers. At the upland site, site #1, eight plants were cross pollinated with another individual plant a minimum of 20 meters, or more, away. Four plants were self-pollinated, and seven plants receiving no human assisted pollination were treated as a control group. All plants in the study were tagged inconspicuously, staked and recorded. At the prairie swale site, site #2, located on federal lands, permission was obtained to hand pollinate five plants and five additional plants were marked as control plants.

Pollen sacs were removed from one individual flower with the aid of toothpicks and placed on the stigma region of another flower. Manipulations and staking were completed at site #1 on June 28th, 1996. Anthesis commenced 19 days later at site #2, and hand pollination at that location took place on July 17, 1996.

M. From, 1997

Results and Discussion

Germination Response

Earliest germination began after five months in cultures for seeds collected at the end of the 1995 growing season. Additional germination continued to occur within those vessels for 15 months. This appears to follow germination patterns typical for other North American native orchid species, (Rasmussen, 1995 and Stoutamire, 1974). Responses vary widely between cultures and the provenance of the seeds from one source plant to another. Germination response ranged from less than 1% to ≈ 20%. Resulting protocorms were recultured on fresh media when they reached 5 mm in length, or longer, and displayed a shoot initial. A protocorm is initially a small white body which develops from an embryo that has imbibed sufficient water, increasing in size until it is capable of rupturing the seedcoat. The protocorm first appears as a translucent sphere that develops tiny hair-like rhizoids which protrude from the protocorm surface. Rhizoids are simple extensions of epidermal hairs which, it is hypothesized, in nature make contact with the mycorrhizal fungus (fungi) which develops a symbiotic relationship with the orchid (Rasmussen, 1995).

Subsequent experimentation with seeds collected in 1996 have reduced initial germination response time to as little as 15 days. Germination rates, however, remain low, even though new pre-culture treatments have synchronized a more uniform germination response and greatly shortened initial response time.

Germination has been obtained on the modified FAST medium and the P/C medium. Further refinements and modifications, both to the previously successful media and pre-culture treatments, are continually being investigated. Culture requirements for *P praeclara* are not available in publications, therefore, each stage of the species' biological development is being recorded for a greater understanding of its requirements.

The very limited amount of plant materials available for this research preclude the staining and viability testing of seeds. This can't be justified since it would involve destruction of seeds which are severely limited in number for this research due to the species' protected status.

Protocorm development proceeds extremely slowly with the majority of individuals. Approximately 0.025 percent of the earliest protocorms demonstrated unusual vigor and precocity. Those individuals displayed shoot, root and tuber initials after 10 months in aseptic cultures. Protocorm mortality appears to be reduced when cultures are given 90 day cold treatment regimes annually, versus cultures kept at 23° ± 2°C continually.

M. From, 1997

Seed Structures

The dry seeds photographed displayed a dark brown structure encasing the embryo (figure #1). The distinctive seedcoat structure is unique to this orchid species. Wet seeds which had been soaking in a bleach solution for one to two hours displayed a somewhat more transparent seedcoat, which in some instances showed liquid solution collecting at a kevelian border momentarily, and then rapidly moving to another collection point at a neighboring border (figure #2). After longer periods of soaking in solution, the liquid flowed past the embryo relatively freely. The bleach solution appeared able to penetrate the seedcoat with progressively greater ease as the soaking time increased in duration (figures #3 #4). Repeated experimentation will be necessary to determine what is the optimal length of time needed in a soaking solution to soften or dissolve the seedcoat in order to foster a maximum germination rate, without doing serious damage to the immature embryo inside.

Hand Pollination Study

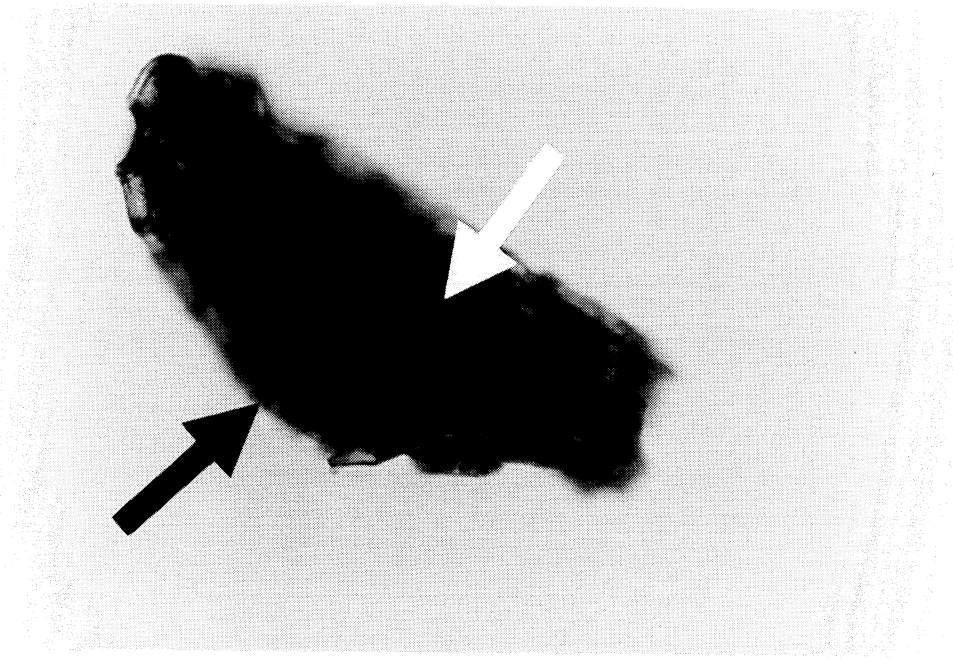
Pollen grains are contained in pollen sacs held together by a sticky substance. Pollen sacs were removed from a flower on one WPFO plant and placed on the stigma region of a flower on another plant located a minimum of 10 meters away or more. Those hand-pollinated plants were marked for seed-set data gathering to be completed in the fall (Fig. 5-7 and table 1). Control plants were marked and left untouched to gather data on whether those control plants would be pollinated by natural pollen vectors. Results from just one year of hand-pollination are inconclusive regarding the relative advantages of this practice as a long-term means to preserve or increase individual WPFO populations. Further research is needed to make decisions about the merit of this manipulation for future fruit-set. Initial data gathered did, however, indicate that self-pollinated plants, and control plants left untouched, produced fewer fully developed fruit capsules than plants which were cross-pollinated by hand.

Some unexpected observations regarding pollen receptivity were obtained, with the manipulated pollination of flowers. Newly opened flowers, or those not quite fully opened or expanded, demonstrated a marked lack of receptivity toward pollinia which were manually introduced to the stigma region, repelling the pollen sacs. Fully expanded flowers which showed no signs of aging were very receptive to the introduced pollen sacs. The pollinia adhered readily to the slightly sticky stigma region. Flowers showing obvious signs of age or damage produced a slick, watery exudate in the stigma region, which repelled the introduced pollen sacs. This may indicate a key factor for successful pollination; the synchronization of the plants' reproductive stages with the life cycle of their natural pollinators, the sphinx hawkmoths, (Sheviak and Bowles, 1986). Timing of insecticide application within proximity of WPFO wild populations

could therefore, impact heavily on the number of available pollen vectors in the area of any given wild population. Future hand-pollination study should be conducted by repeated visits to a site during flowering season, in order to hand-pollinate flowers at a time when successful pollination is most likely to be achieved. Each flower is capable of lasting 7-10 days, (Pleasants and Moe, 1993 and Fritz, 1993).

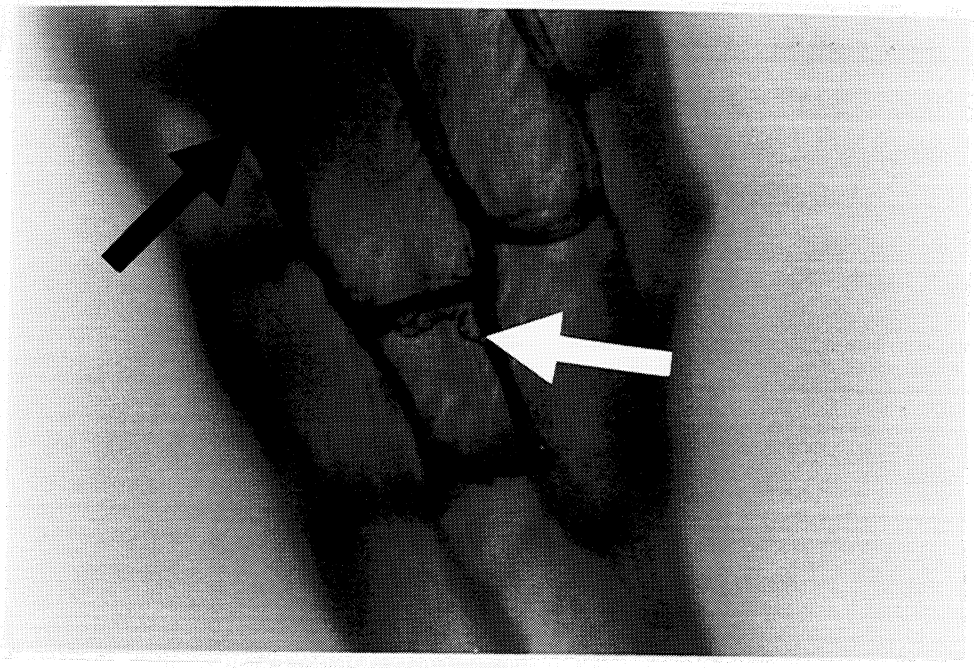
Data on fruit set were gathered at site #1 on September 13, 1996 and data were recorded at site #2 on September 20, 1996. Data at both sites were recorded when fruits had developed fully and capsules began to show some browning areas on the fruit exteriors. Average fruit set when flowers are pollinated by natural pollen vectors on the Sheyenne National Grasslands is approximately 30%, (Pleasants and Moe, 1993). Average fruit set at sites 1 and 2 in this study were lower. It is hypothesized that fewer natural pollinators may be visiting these western populations which are widely scattered and disjunct.

Figure #1



A *Platanthera praeclara* seed photographed in the dry state at 200x. Note the dark opaque seedcoat (black arrow). The embryo within is barely visible (white arrow).

Figure #2



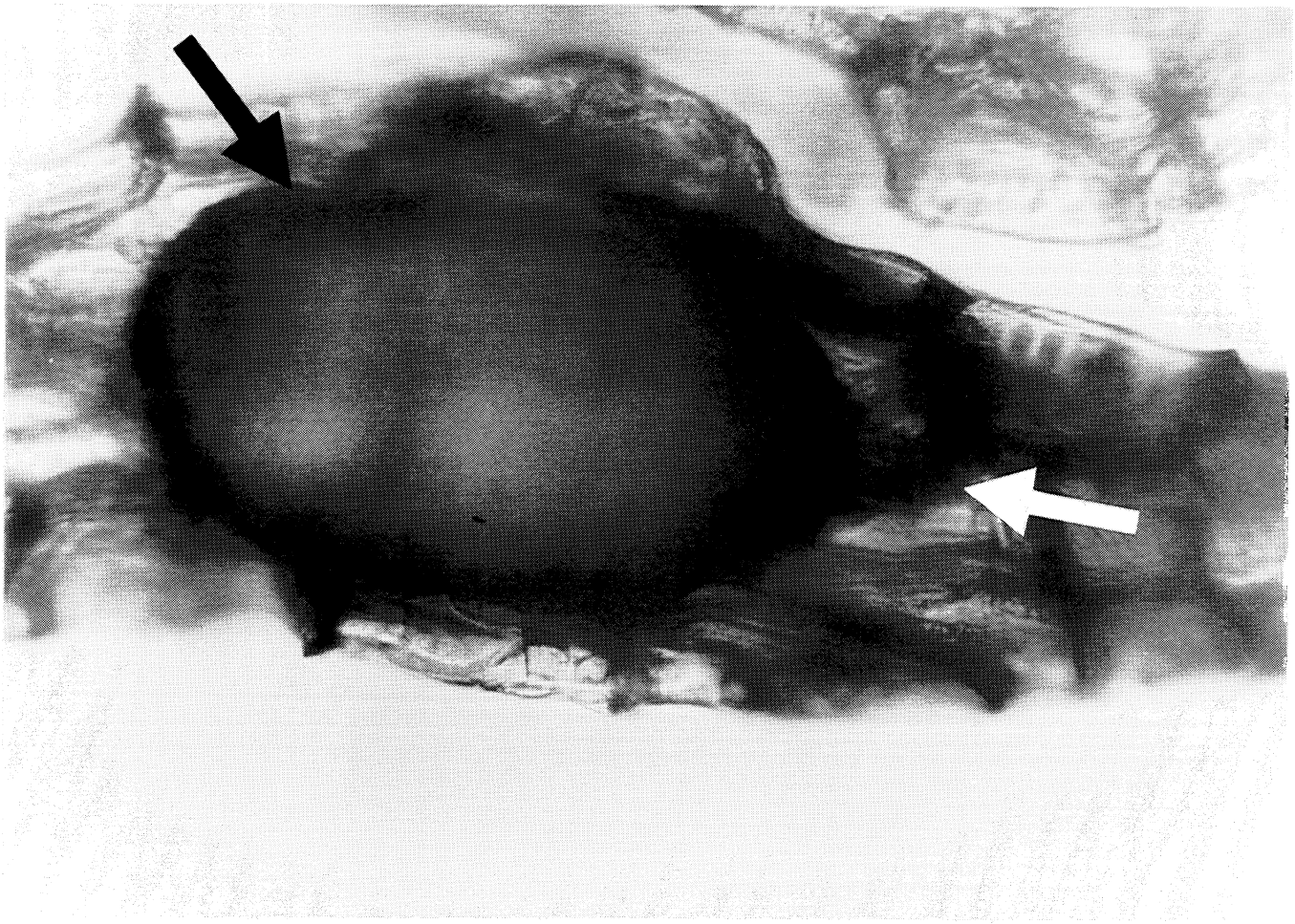
A *P. praeclara* seed soaked in a bleach solution and photographed in the wet state at 400x. Water droplets have accumulated at a kevelian border before passing through to the neighboring segment of the seedcoat (white arrow). Kevelian border structures hold the embryo inside of the seedcoat (black arrow).

Figure #3

A *Platanthera praeclara* seed at 200x after a one-hour soak in sodium hypochlorite solution. Note the slight transparency to the seedcoat in some areas (white arrows).

M. From, 1997

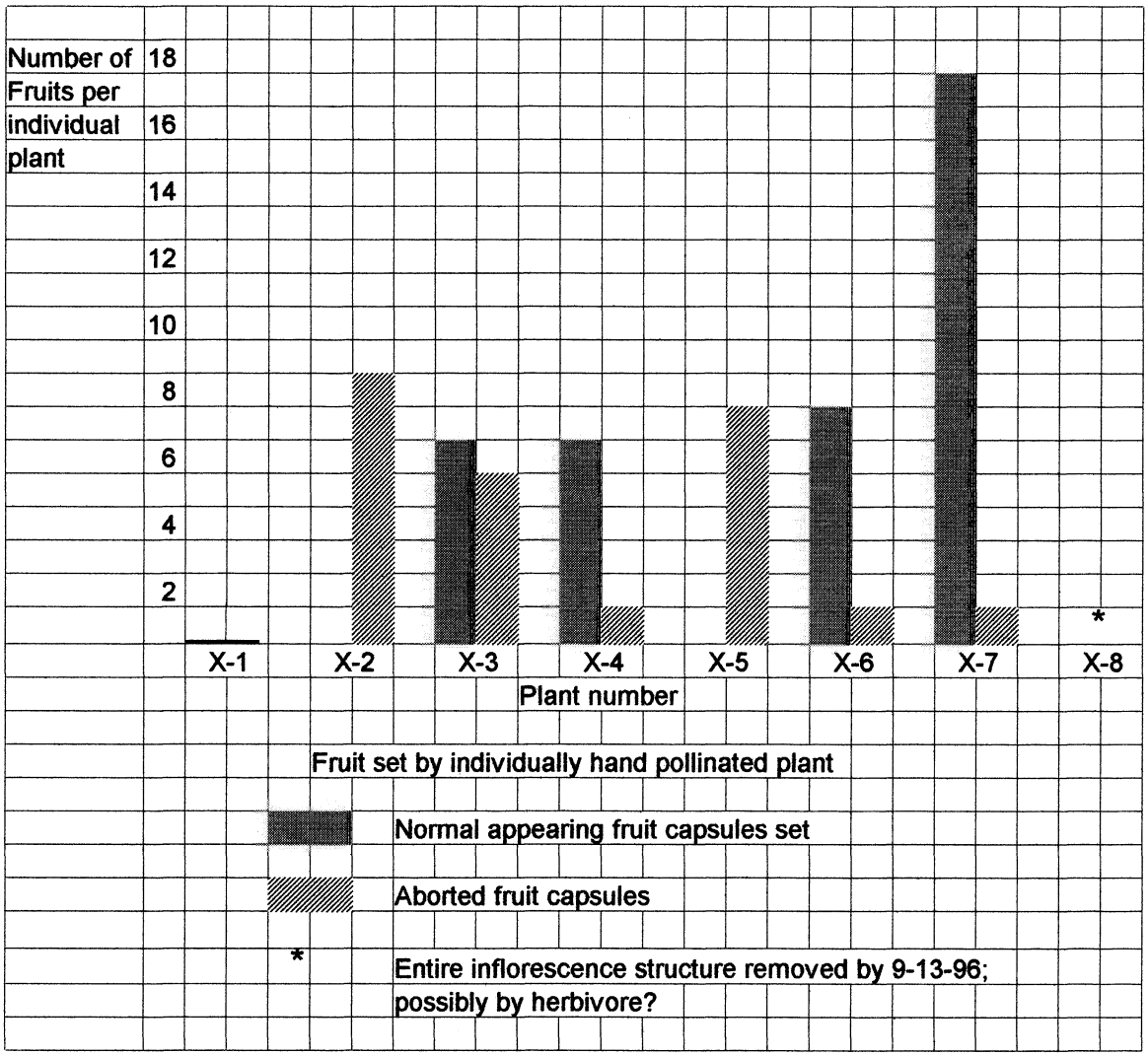
Figure #4



The same *P. praeclara* seed as in figure #3, at 400x. Note the prominent embryo mass within the seedcoat (black arrow). The suspensor area is clearly visible (white arrow).

M. From, 1997

Figure #5 **1996 Cross pollinated plants site #1**

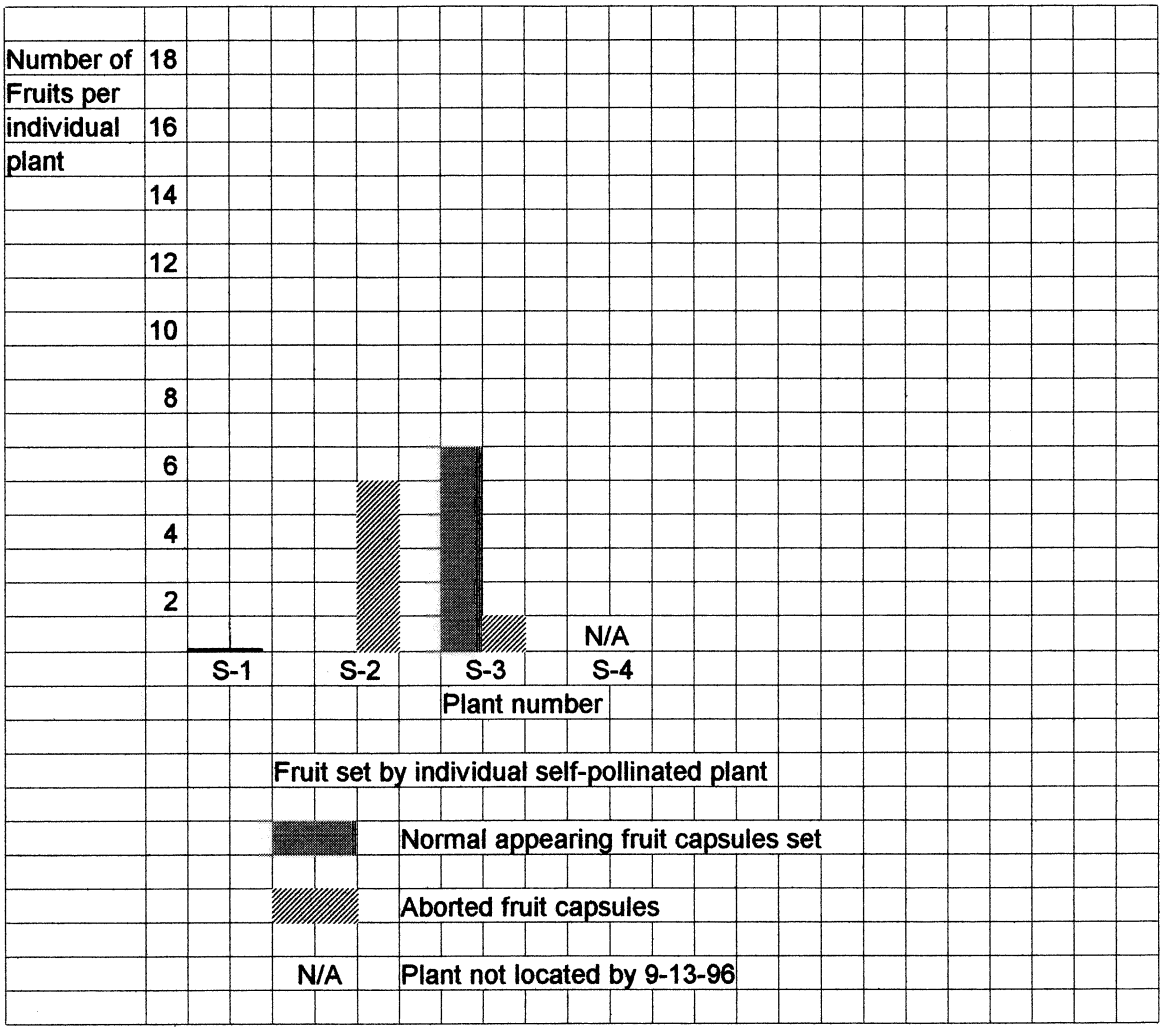


Total available cross pollinated plants on 9-13-96 = 7
Total number of available manipulated flowers = 35
Total number of normal-appearing inflated fruits = 36 **
Total number of aborted fruits = 23

** Natural pollen vectors are hypothesized to have randomly pollinated plant X-7 in addition to human assisted pollination.

M. From, 1997

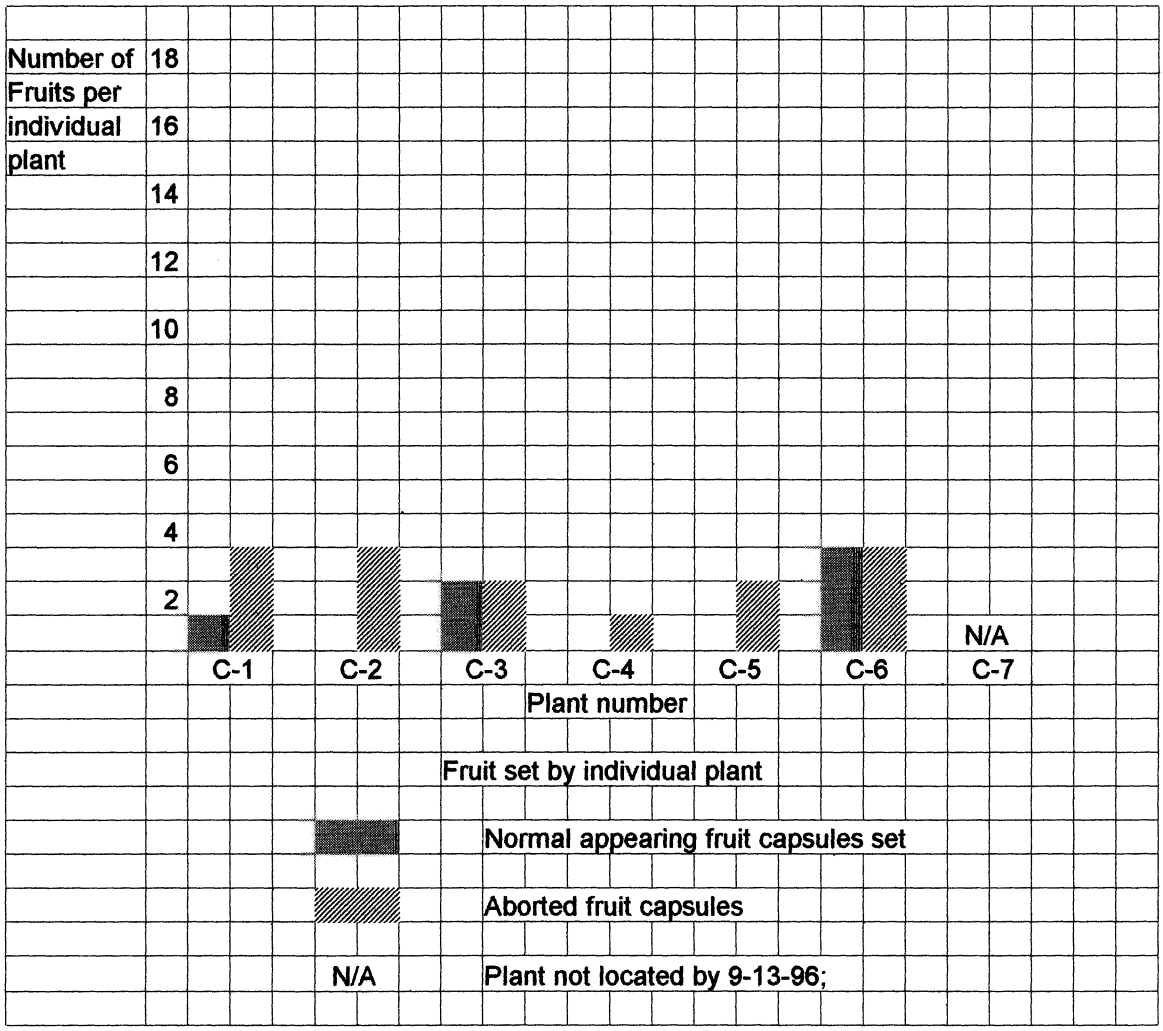
Figure #6 **Manipulated Self-pollinated plants. Fruit set 1996. Site #1**



Total available self-pollinated plants on 9-13-96 = 3
Total number of available manipulated flowers = 15
Total number of normal-appearing inflated fruits = 6
Total number of aborted fruits = 6

M. From, 1997

Figure #7 **Control plants. No manipulated pollination, 1996. Site #1**



Total available control marked plants = 6
Total number of normal-appearing fruits = 6
Total number of aborted fruits = 14

M. From, 1997

* Table 1 **1996 Pollination Study at Site #2**

Cross-pollinated by hand

Plant #	# of flowers X-Pollinated	# of seedpods set by 8-13-96	# of mature seedpods at 9-20-96
#X-1	7 of 10	0	0
#X-2	8 of 10	3	3
#X-3	7 of 16	12	10
#X-4	8 of 12	5	0
#X-5	3 of 4	4	3

Control Plants

Plant #	# of flowers present on 7-17-96	# of seedpods set by 8-13-96	# of mature seedpods at 9-20-96
#C-1	unavailable	0	0
#C-2	6	2	2
#C-3	12	10	0
#C-4	12	7	6
#C-5	unavailable	1	0

* (Unpublished data 1996. Valentine National Wildlife Refuge)

#X-1 entire inflorescense clipped off (herbivore?)
#X-4 plant not found on 9-20-96
#C-3 entire plant damaged at observation date 9-20-96
#C-5 plant not found on 9-20-96

Cross pollinated
Total number of available cross pollinated plants on 9-20-96 = 3
Total number of available manipulated flowers on 9-20-96 = 18
Total number of normal-appearing inflated fruits on 9-20-96 = 16
Control
Total number of available control plants on 9-20-96 = 3
Total number of normal-appearing inflated fruits on 9-20-96 = 8

Conclusions and Recommendations

This research has demonstrated that it is possible to germinate *P. praeclara* seeds and sustain early protocorms in asymbiotic cultures for twelve months, or longer. In some cases, certain protocorms have been in culture for less than twelve months, but more recently cultured seeds have demonstrated a higher germination rate than the earliest cultures. This may be attributed to several factors, among which may be a necessary after-ripening period for the seeds subsequent to their maturation on the plants, longer refrigeration of the dry seeds before culturing, improvements made in disinfestation procedures before culturing, media component changes made in the later seed-sowings, or still unidentified factors within the seeds themselves. It should be pointed out that the seeds used in each seed-sowing came from the same location, collected on the same date and were all drawn from the seed lot when cultured. Germination rates are still low, but are improving with each successive seed-sowing.

Protocorms produced root initials in greater lengths and greater numbers after given either a 60 or 90 day cold treatment. Roots 1.5 cm in length, or longer, displayed tendencies of contractile roots, growing downward into the medium and pulling the plantlet's crown area down into direct contact with the medium or up to 3 mm below the surface. *P. praeclara* protocorms do not each produce a tuber in the first 12 months in culture. Those which produced a tuber in the first year had fewer roots, but those roots had a mean diameter of 3 mm, versus 1-2 mm in diameter on protocorms which did not produce an early tuber.

Each individual culture sown with seeds is coded for location and date of seed collection. Seeds from plants which were manipulated by human assisted pollination are labeled to track the germination response and developmental morphology of resulting protocorms and subsequent plants. All plants produced to date have demonstrated normal physiology. Conservation of the gene pool involves preservation of genetic material which is true to the species' wild form.

The number of annual frost free days differ between the two sites. Annual frost free days for site #1 are 200 and annual frost free days at site #2 are 160 days. At site #1 the number of days between pollination and time of dehiscense was 78 days. The days between pollination and dehiscence was 65 days at site #2. Plants which had been hand pollinated and possessed at least one or more inflated seed capsule, persisted in a vegetative state longer than plants which were subject to random pollination by natural pollen vectors. Those plants which produced twisted, deflated fruits were totally brown by late September at both sites.

M. From, 1997

Hand-pollination was limited in scope by the number of available plants; a relatively small number of individual plants. Not all of those individuals were available at the time data were gathered late in the season. Therefore, data are inconclusive with regard to the feasibility of implementing this as a routine strategy for increasing seed-set in wild populations. Further study is required with a larger number of plants in the future, so that it can be ascertained whether this is a desirable practice. Caution is being exercised because seed production is a costly function for most plants, in terms of the plant's total energy reserves. This study is being carried out with the highest regard for the continued health, viability and presence of the Western Prairie Fringed Orchid populations within the state, as well as the Great Plains Region in general.

Much more research on this orchid species is planned for the future. This research project will continue by utilizing seed collection allowed from future growing seasons. Further refinements are being implemented both in the disinfestation and the culturing techniques with the fresh seeds. Data-gathering will continue with established cultures from 1995 and 1996 growing seasons.

Assessment is needed of the threat of extinction to current populations of the Western Prairie Fringed Orchid, *Platanthera praeclara*. Future research into its propagation for possible reintroduction efforts should recognize several important factors: 1) the possibility that a single threat could be capable of causing 100% mortality in a sufficient number of the very small populations (those with 10 or fewer plants) scattered throughout the state, so as to pose a real threat to the species' viability within that state or region, 2) the likelihood of natural fluctuations in populations to synchronize at a low point that could threaten the species' viability, 3) the genetic viability and variability of the species in a given region, and 4) the likelihood that suitable habitat will continue to be available. These issues are particularly important in areas where intensive cultivation practices and their accompanying heavy uses of herbicides, pesticides and fertilizers, or heavy grazing practices and repeated mowings are carried out, which can each dramatically affect WPFO fruit-set.

Although simultaneous floods, fires or disease pose a relatively small threat to all populations throughout the orchid's range, one population may represent a considerable percentage of the species' genetic base in sparsely populated regions. Therefore, the loss of even one, or a few small populations simultaneously, could drastically reduce the species' total genetic variability. It is currently unknown how much genetic exchange occurs by natural pollen vectors among the individual sites, and future research may point to the possibility that manipulated crossings between populations may be necessary to maintain species viability, since populations are now often found in non-contiguous

colonies. One possible future remedy may be to reintroduce new populations in protected areas within the species' historic range.

The occurrence and magnitude of the species may overall be relatively high, particularly in the northeastern portions of its range within the Great Plains Region. However, this may be of little consequence in the western and southern portions of its range if individual populations do not remain viable over the long term, and diminished populations no longer have any genetic exchange between their disjunct locations. Micropropagation, as a possible source of WPFO plants in the future, may become increasingly important for the species' preservation and continued presence in the western portions of the orchid's range.

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Short Report on the *In Vitro* Propagation and Anatomical Studies of Temperate Orchids (Orchidaceae).

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In recent decades the rate of extinction of plant and animal species has been alarmingly high. The damaging effect of human activity on natural ecosystems, and particularly the rapid pace at which species have disappeared has become a growing public concern. The extinction of these species is a consequence of the accelerated spread of humans into remote habitats, the introduction of toxic chemicals into the environment and the exploitation of endemic animals and plants (Beckner, 1979; Wochok, 1981; Ronse, 1989; R. Eszéki and Szendrák, 1992; Affolter, 1997).

There is a worldwide growing interest in nature conservation and the obvious need to conserve and save plant species close to extinction. Therefore the IUCN (International Union for Conservation of Nature and Natural Resources/ The World Conservation Union) stated in its Conservation Strategy as some of the most important goals for botanical gardens, universities and other institutions related to the area, the conservation of rare (sometimes threatened or endangered) plant species, the preservation of their native habitats, development of new proliferation/propagation techniques and reestablishment of the species into previous native habitats or into similar protected environments. The first Action Plan by IUCN for plants was prepared especially for orchids (IUCN/SSC Orchid Specialist Group, 1996). New propagation techniques serve as a tool not only for producing plant material for reintroduction but could also provide plants available for the public, thus lessening collectors' temptation to gather them in the wild (Ronse, 1989; Stewart, 1992). In recent years several different projects for rare and/or endangered plant species were established at the Department of Horticulture, University of Nebraska-Lincoln, including extended research with several European and North American temperate orchids and some carnivorous plants and cacti (contact person: Dr. Erika Szendrák), with the western prairie fringed orchid (*Platanthera praeclara*) and

other North American terrestrial orchid species (contact persons: Margaret From and Dr. Paul E. Read), with the blowout penstemon (*Penstemon haydenii*, contact persons: Dr. James Stubbendieck, Dr. Jay Fitzgerald and Julia Lamphere) and with the American chestnut (*Castanea dentata*, contact persons: Dr. Paul E. Read, Dr. Erika Szendrák and Virginia I. Miller) with promising results.

Being one of the most specialized groups of the plant world, the members of the Orchidaceae provide fascinating phenological, anatomical and histological facts to scientists. This family is among the most highly evolved plant families in the Monocotyledonopsida which is notable for its unique flower structure, specialized life cycles and modes of proliferation, among others. The number of species in the family is about 20,000, and even in this number there is a great variability among the different species and a huge diversity in structural details.

Most of the temperate native terrestrial orchids are endangered/threatened species (Beckner, 1979; Arditti and Ernst, 1993; Stewart, 1992; IUCN/SSC, 1996) and the propagation of these species from seeds poses specific problems. Orchid seeds are extremely small and the embryo is in a rudimentary stage. In most cases the seed is actually devoid of endosperm and they need microscopic fungi in a symbiotic relationship in nature for germination.

Some results of an extended research project (Szendrák, 1997) were presented, and here a short summary is provided to describe the most important observations. Seeds from 52 different species of temperate terrestrial orchids were disinfested with a 10% calcium hypochlorite solution for 10 min and sown on a modified FAST medium (Fast 1976; Szendrák and R. Eszéki, 1993). After sowing, the cultures were kept in the dark at 10-12°C for four weeks. The cultures then remained in the dark, but the temperature was raised to 25-26°C until germination occurred. Thereafter cultures required alternating seasonal temperatures: 25-26°C from the beginning of April to the end of September and 17-19°C from October to March. Growth stages, including the date of seed and embryo imbibing, opening of the seed coat and earliest protocorm stage, were recorded and compared among the different species. Seed germination was considered successful when

small white protocorms covered with translucent hairs and possessing a definite apical dome were observed. This stage also varied significantly among species. The shortest time for complete germination was observed in the genus *Orchis* (~28 days); and the longest in the genus *Cypripedium* (~250-300 days), but it generally ranged around 50 to 90 days. For the development of the young plantlets natural dispersed light and prevailing day-length were favorable. Twenty-five different orchid species were successfully grown with this method and after two to three years of aseptic culture they were suitable for transfer *ex vitro*.

The fascinating anatomical structures and developmental patterns of several temperate orchid species were observed. Samples of *Corallorhiza odontorhiza* (Will.) Nut., *Cypripedium acaule* Aiton, *Cypripedium calceolus* L., *Cypripedium californicum* A. Gray, *Dactylorhiza fuchsii* Soó, *Dactylorhiza maculata* (L.) Soó, *Dactylorhiza majalis* (RCHB.), *Galearis spectabilis* (L.) Rafin., *Himantoglossum hircinum* (L.) Spreng., *Ophrys lutea* Cav., *Orchis mascula* L., *Orchis morio* L., *Platanthera bifolia* (L.) Rich., *Platanthera hyperborea* (L.) Lindley, *Platanthera praeclara* Shev. & Bow., *Spiranthes cernua* (L.) L. C. Rich., *Spiranthes vernalis* Eng. & Gray were legally field collected, received from botanical gardens, taken from herbarium specimens or were harvested from *in vitro* cultures initiated from seeds. Plant material samples from several different organs were observed macroscopically and also by scanning electron microscopy (SEM).

The first visible step of germination was the opening of the seed coat, when the first few white cells became visible. After a few weeks the apical meristem appeared. The young protocorm was covered with numerous translucent rhizoids. In the last stage of germination the first true leaf and the first root started to develop. Structure of the mature organs and tissues were also examined. New information was found and was reported in the presentation about the structural details of roots with orchid-type mycorrhiza and stored materials (calcium oxalate crystals and starch), leaf/stem stomatal structure and anatomical details of the generative organs and ovule/seed development.

Anatomical details (on SEM images as slides or prints) were presented about *Platanthera praeclara* at the Western Prairie Fringed Orchid PHVA workshop about the following plant parts:

- seed (morphology and structure);
- leaves (including stomata, trichomes/epidermal hairs, accumulated starch grains and calcium oxalate crystals);
- vegetative parts of the flower (including lip surface, spur inner and outer surface)
- generative parts of the flower (including pollinarium, ovary and embryo structure)
- young fruit (with the ripening seeds)

Plant material for the SEM observations on *Platanthera praeclara* was provided by the Nebraska Statewide Arboretum and by the Charles E. Bessey Herbarium, University of Nebraska-Lincoln. Their contribution is greatly appreciated.

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Study Proposal for

Genetic Analysis to Determine Variation for Subpopulations of the Western Prairie Fringed Orchid, *Platanthera praeclara*

Jennifer Hancock, University of Northern Iowa and Henry Doorly Zoo
Western Prairie Fringed Orchid Population and Habitat Viability Assessment Workshop
Mahoney State Park
27-30 April 1997

Determining the genetic diversity between the subpopulations of the western prairie fringed orchid is an important question which must be answered for proper management of the species. Currently, differences at the organismal and molecular level between populations are not fully understood. Recognizing these differences may be important on determining if certain populations are being affected by habitat fragmentation and isolation.

Although previous research involving allozyme analysis exhibited typical levels of genetic diversity found for most orchid species, protein analysis did not provide the necessary information to accurately distinguish the populations or species of orchids. This study will combine DNA fragment analysis utilizing both randomly amplified polymorphic DNA (RAPD) and microsatellite nuclear DNA markers with nuclear DNA sequencing data. Microsatellite nuclear DNA markers developed for this study will also be utilized to reliably identify individuals which will aid in studies of dormancy.

Obtaining this information in as short of time as possible is a high priority. Success and speed of this project may be greatly aided by the participation of a number of groups in sampling their sites during other studies involving the western prairie fringed orchid or in providing access to these sites for sampling purposes by the primary investigator. For those individuals who wish to aid in sampling of their populations, I will request that twenty-five individuals be sampled where possible. A 1-2 inch leaf cutting from plants, either in the vegetative or flowering state, will provide enough material for DNA extractions. I will provide packets of silica gel desiccant for storage of the tissue. Supportive information will also greatly enhance the value of this study, such as accurate location of the individuals sampled and the distances in between individuals. This will help in analysis of diversity within populations and in future studies to provide accurate identification of individuals and their dispersal patterns.

Current Status of Research for

Genetic Analysis to Determine Variation for Subpopulations of the Western Prairie Fringed Orchid, *Platanthera praeclarea,* and the Eastern Prairie Fringed Orchid, *Platanthera leucophaea*

Jennifer Hancock, University of Northern Iowa and Henry Doorly Zoo

Tissue samples have been collected for many populations of the western prairie fringed orchid across their range. Populations sampled in Nebraska include Dickerson Prairie, Dieken Prairie, Twin Lakes Natural Area, two Cherry County populations, Kubicek Prairie, and Nine Mile Prairie. The assistance of Gerry Steinauer, Tim Knott, and Mark Dietz in these collections is greatly appreciated. The Little Tark Prairie, Helton Prairie, and Tarkio Prairie Natural Area in Missouri are being sampled by Dr. David Ashley. A population of the eastern prairie fringed orchid has been collected from Baldwin Marsh in Jackson County, Iowa, however the western prairie fringed orchid is not yet in bloom in the populations I am sampling. Sheeder Prairie and Steele Prairie will be visited this summer in order to complete an additional project in Iowa next summer. Minnesota populations have been sampled by Nancy Sather, Welby Smith and Brian Winter. Wisconsin populations of the eastern prairie fringed orchid have been sampled by David Kopitzke, Ursula Petersen, and Quan Bahn. The Manitoba population will be sampled by Karen Johnson. Based on the results of the genetic analysis of these populations, further sampling will take place during the summer of 1998. Further study of the eastern prairie fringed orchid population will include the easternmost edge of its range in Virginia, Maine, and Ontario and the central populations in Ohio.

Because the Minnesota populations have a larger base of permanently marked individuals, they will most likely be the source of the western prairie fringed orchid DNA used in the plant identification study. If an individual that was collected this year does not reappear next year but does reappear at a later time, tissue from that individual will be collected and its DNA extracted. Microsatellite nuclear DNA markers that have been developed for the determination of the genetic variation will be used to determine if it is indeed the same individual. If it is found to be the same individual, dormancy exists in this plant. If, however, it is a different individual, other samples from individuals which had possibly reappeared after a year or more of being absent must be sampled and run to make a valid determination of the existence or non-existence of dormancy in the western prairie fringed orchid.

We currently have two genomic DNA libraries developed for the western prairie fringed orchid. The first library is constructed from total genomic DNA and is basically a collection of clones, each containing a small unique fragment of nuclear DNA from one individual orchid (each piece of DNA is between 200 and 1000 nucleotide bases in length). This library has been screened with three probes which are GT, GATA, and AATG repeats. One microsatellite marker has already been developed from this library. From these cloned fragments, we have noted that orchid DNA sequence has a higher percentage of AT (adenine and thymine) nucleotides. The AT rich orchid DNA makes it difficult to find microsatellites with the probes that we have been using from the first library. Using AT probes creates a problem because the probe binds to itself at the normal hybridization temperature. Although increasing the temperature will denature the probe, the higher temperature would prevent it from hybridizing to the DNA filters. Another library has been developed utilizing PCR and cloning to enrich the number of clones containing microsatellites. The probe-specific libraries will be screened with eight different florescent probes to increase the number of markers for the study.

The project is currently on schedule with some DNA sequencing of nuclear DNA beginning this summer at the zoo. I will begin RAPD analysis in the fall using DNA samples from this summer's collections. Further development of microsatellite markers will continue when I return to UNI in the fall. Analysis using microsatellites will begin in January 1998 when I return to the zoo and will continue during the following summer.

POPULATION AND HABITAT VIABILITY ASSESSMENT (PHVA) FOR THE WESTERN PRAIRIE FRINGED ORCHID (*Platanthera praeclara*)

Eugene Mahoney State Park
Ashland, Nebraska
27-30 April 1997

Final Report

Appendix II.
Project Proposals

Genetic Analysis to Determine Variation for Subpopulation of the Western and Eastern Prairie Fringed Orchids Across their Range

Jennifer Hancock, student, Master of Environmental Science
University of Northern Iowa
Henry Doorly Zoo

Dr. Douglas L. Armstrong, Research Director
Henry Doorly Zoo

Total requested: $14,457.50
Duration of project: Two year

PROJECT SUMMARY

Genetic variation within and between populations of the western prairie fringed orchid, *Platanthera praeclara*, and the eastern prairie fringed orchid, *Platanthera leucophaea,* will be examined to establish the genetic diversity across their range, the level of gene flow between isolated populations, and the relatedness between the two species. Limited variation both within and between populations was detected in these two species using allozyme data (Pleasants and Klier 1995). Recent molecular techniques, however, are available which can identify variation not detectable utilizing protein analysis (Sites and Davis 1989). By combining DNA fragment analysis using microsatellite nuclear DNA markers and randomly amplified polymorphic DNA (RAPD) as well as nuclear DNA sequencing data, variation at the nucleotide base level can be detected. Microsatellites have a high rate of mutation compared to other markers; and therefore, provide the high rates of polymorphism necessary for population comparisons. The microsatellite markers developed in this project will also be utilized in subsequent studies identifying individual plants for life history studies. RAPD analysis provides a fast and inexpensive comparison of genetic variation at the nucleotide base level. Both techniques will be used, along with nuclear DNA sequencing, to give a more accurate picture of the diversity within the prairie fringed orchid genome.

PROJECT DESCRIPTION

A) INTRODUCTION

Due to habitat destruction for industrialization and agricultural purposes, less than one percent of the native prairie habitat of the Great Plains remains intact today. Species loss on the fragmented prairie remnants is occurring at an alarming rate. The western prairie fringed orchid, *P. praeclara* Sheviak and Bowles, and eastern prairie fringed orchid, *P. leucophaea* (Nutt.) Lindl., are federally listed threatened species, whose range occurs in the tall grass prairies of the central United States (U.S. Fish and Wildlife Service, 1989). They are also among only a few species or orchids characteristic of this region (Sheviak and Bowles, 1986). The two species have primarily allopatric ranges with the more western *P. praeclara* occurring primarily in the Missouri River drainage basin and the eastern counterpart, *P. leucophaea* occurring in the upper Mississippi River drainage basin (see figure 1).

Metapopulations of the western prairie fringed orchid occur in Manitoba, Minnesota, and North Dakota. Population sizes in Iowa, Nebraska, Missouri, and Kansas tend to be smaller (1-75 individuals) and more isolated. Eastward margins of the eastern prairie fringed orchid seem to have retreated toward the west since the close of the Wisconsonian glaciation which has left disjunct populations in the east (i.e. Virginia, Maine, New Jersey). Metapopulations of the eastern prairie fringed orchid occur in the southern Lake Michigan region (Bowles, 1983).

B) RATIONALE AND SIGNIFICANCE

Based on county records, a 60% decline in *P. praeclara* and a 70% decline in *P. leucophaea* have been observed (Harrison 1989). This dramatic decline in population numbers has primarily been caused by habitat loss due to conversion of prairies to agricultural land and highly specific pollinator requirements. The western prairie fringed orchid is pollinated by only a few species of hawkmoths of the family Sphingidae whose numbers have been reduced due to isolated habitats and pesticide use (Fritz 1993).

The drastic decline in fringed orchid population numbers poses a threat to the genetic variability among the populations. As genetic variability decreases, populations become less able to adapt to change, threatening their long term persistence in the face of environmental change and catastrophic events. The short term population fluctuations characteristic of the fringed orchids have made it difficult to determine whether individuals per population have been declining; however, the small numbers of individuals characteristic of the majority of the fringed orchid populations may lead to reduced genetic variation within individual populations (Pleasants and Klier 1995).

Population studies have been hindered due to gaps in the basic understanding of the life history of the fringed orchids. The rarity of the fringed orchids and the population fluctuations characteristic of the two species make them difficult to study. The life span of individuals has been heavily debated based on conflicting experimental results (Hull-Sieg, Sather, Bowles 1983). It is uncertain whether these plants undergo a dormant phase, which is typical of many orchid species, due to the shift in location of marked individuals over several years. The restriction on unearthing plants to determine if the plant is in a dormant phase has made genetic markers a useful tool to identify an individual plant over a number of years; I plan to determine if a plant which becomes absent for a year or more is the same individual which was once present. Genetic markers will also provide information about the frequency of clones in these orchids. All of this information is vital for accurate population modeling used to determine the viability and status of the fringed orchids (Pavlik 1993).

Recovery plans for threatened and endangered plants must take into account the population demography applied at the community and landscape levels. The fate of each population of the fringed orchid depends on its genetic, demographic, and environmental history dynamics. Four factors can lead to extinction. Widespread catastrophes and local environmental stochasticity can cause the extinction of entire populations at once because they affect all members of a population. Demographic and genetic stochasticity affect individuals; however, when population sizes are small there is an increased chance of the entire population becoming extinct (McEachern *et al*, 1993). These small populations also may not attract enough pollinators leading to reduce seed production. Because sexual reproduction is necessary for long term persistence of the fringed orchid populations, it is imperative that plans for population

preservation and be developed based on the genetic analysis of existing populations (Pleasants and Moe 1993).

The Population and Habitat Viability Assessment workshop (held by the Conservation Breeding Specialist Group (SSC/IUCN) during April 1997) asserted that determining the genetic diversity remaining in the populations and providing accurate identification of individuals to be two of the most important goals in preserving the fringed orchids. The only previous population genetics study on these two plants (Pleasants and Klier 1995) utilized allozyme analysis which showed little variation both within and between populations. Allozyme analysis presents a limited view of the genome because it examines differences at the protein level. A more accurate description of variation within and between the populations will be provided by examining differences at the nucleotide base level. Microsatellite nuclear DNA markers and randomly amplified polymorphic DNA (RAPD) analysis will be utilized in this project along with nuclear DNA sequencing. The resulting data will be used to determine the diversity of the western and eastern prairie fringed orchids and the relatedness between the two species. Microsatellite nuclear DNA markers will be used to accurately identify individuals and subsequently determine the longevity of individuals.

C) EXPERIMENTAL PLAN

Sampling

Samples of the fringed orchids have already been either provided to me or collected by myself from populations in Manitoba, Minnesota, Nebraska, Iowa, Missouri, and Wisconsin. A one to two inch leaf cutting from each individual sampled was placed in silica gel desiccant to dry the tissue. Where possible, twenty five individuals were collected per population. Populations in Maine, Virginia, Ontario, Michigan and Ohio will be collected during the summer of 1998.

Microsatellites

Microsatellites are short tandem repeats of nucleotide bases which are found interspersed throughout the genomes of prokaryotes and eukaryotes. They have a higher rate of mutation than other nuclear coding regions; and therefore, provide an excellent molecular technique to identify variation among organisms. Microsatellite markers are also presented as a codominant trait, displaying both the maternal and paternal contribution to the plant. Currently, no microsatellite nuclear DNA markers are available for orchids, therefore, I am developing my own primers which are specific for the western prairie fringed orchids, and should be compatible with other orchids.

I currently have two genomic DNA libraries developed for the western prairie fringed orchid. The first library is constructed from total genomic DNA and is basically a collection of clones, each containing a small unique fragment of nuclear DNA from one individual orchid (each piece of DNA is between 200 and 1000 nucleotide bases in length). This library has been screened with three probes constructed from the following nucleotide repeats: GT, GATA, and AATG. One microsatellite marker has already been developed from this library. These clones are sequenced and PCR products are designed which flank the microsatellites. From these fragments, I have noted that orchid DNA sequence has a higher percentage of AT (adenine and thymine) nucleotides. Thus, the AT rich orchid DNA makes it difficult to find microsatellites with the probes that we have been utilizing on the first library. Using AT probes creates a problem because the probe binds to itself at the normal hybridization temperature. Although

increasing the temperature will denature the probe, the higher temperature would prevent it from hybridizing to the DNA filters. Another library has been developed utilizing PCR and cloning to enrich the number of clones containing microsatellites. The probe-specific libraries will be screened with eight different florescent probes to increase the number of markers for the study.

These clones will then be sequenced and primers designed. The number of repeats within the microsatellite tends to differ between individuals and populations due to slippage during replication; therefore, the PCR products will be slightly different sizes. These products will be run on the LI-COR automated sequencer which uses florescent dyes to distinguish between bases and comparisons can be made among individuals within and between populations. I plan to develop a minimum of twenty microsatellite nuclear DNA markers for this project.

RAPD analysis

RAPD analysis, another technique I will utilize, is a fast and inexpensive method of comparison. Since RAPD analysis is based on a dominant marker system, the presence or absence of alleles, laboratory technique is essential for reproducibility and accuracy of the results. Although microsatellites are a valuable tool for analysis of genetic variation, primer design is time consuming and expensive. RAPDs sidestep primer design by using a large set of random primers. Using PCR, even random primers will anneal with some probability. These products are electrophoresed on an agarose minigel and stained with ethidium bromide. The gel can then be photographed using an ultraviolet light source to determine banding patters. These products may not occur on every chromosome or in every individual. By using a large number of primers which produce polymorphic banding patterns, comparisons can be made between and within populations.

Nuclear DNA sequencing

The small white lady's slipper orchid, *Cypripedium candidum*, is currently being sequenced using nuclear DNA (Anne Antlfinger, University of Nebraska at Omaha). This species will be used as the outgroup for the fringed orchid. Comparisons of nuclear DNA sequence will be made using the PAUP (version 3.1, Swofford). Through combining sequencing data and fragment analysis, high quality comparative data can be obtained. Sequencing also provides a reliable genetic distance measure between species and subspecies. Complemented by microsatellite analysis, the sequence data will contribute important information to the project, combining high resolution with broad coverage of individuals.

E) LITERATURE CITED

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TIME SCHEDULE

Project duration ----------> 2 years

Summer 1997	Collection of tissue samples (Manitoba, Minnesota, Nebraska, Iowa, Missouri, Wisconsin) and DNA extraction Nuclear DNA sequencing Microsatellite nuclear DNA primer development
Fall 1997	RAPD analysis
Spring 1998	Continuation of microsatellite primer development Microsatellite analysis
Summer 1998	Continue sampling (Ontario, Maine, Virginia, Ohio, Michigan)
Fall 1998	Completion of RAPD and microsatellite analysis

FACILITIES AVAILABLE

Samples will be analyzed by myself at two separate locations. RAPD analysis will be completed at the University of Northern Iowa in Cedar Falls. The plant systematics laboratory is equipped with a thermal cycler capable of running up to 150 PCR samples at a time. The lab also contains the necessary cooling units, a -20°C cooler and a 4°C cooler. Minigel rigs, florescent light source, and photography equipment is also available. The molecular biology laboratory at the University also contains an oligonucleotide synthesizer for creating primers and computers containing software for analyzing genetic data.

The genetics lab at the Henry Doorly Zoo is equipped with two automated sequencers; the ABI 373 is used for primer development and nuclear DNA sequencing and the LI-COR 4000 is used for running individual orchid PCR products amplified with microsatellite nuclear DNA primers. Two Techne thermal cyclers and a Perkin Elmer thermal cycler are available for PCR, ligations, and cold-testing primers. Cooling equipment includes a 4°C stand-up cooler, a 4°C walk in cold room, two -20°C chest freezers, a -20°C walk-in freezer, and two -80°C coolers. The freezer systems equipped with an alarm system to maintain sample integrity. Other equipment in the lab includes an electroporator, a Speed-Vac, water baths and shakers, heating blocks, three incubators, photo-equipment, a fluorometer, and a refrigerated centrifuge.

Two other laboratories are available for my use: radioactive work is done at the University of Nebraska at Omaha's Medical Center, and the California State at San Marcos has created a second western prairie fringed orchid library for my project.

Using Genetic and Demographic Considerations in the Sampling and Reintroduction of the Western Prairie Fringed Orchid, *Platanthera praeclara*

Jennifer Hancock, student, Master of Environmental Science, University of Northern Iowa
Steve O'Kane, Ph.D., Department of Biology, University of Northern Iowa
Ed Louis, DVM, Ph.D., Genetics, Henry Doorly Zoo

in conjunction with the Iowa Department of Natural Resources

Project Summary

Two large representative populations of the western prairie fringed orchid will be sampled in order to determine the genetic variability within the populations. Based on analysis of these populations, recommendations will be made as to appropriate donor sites to obtain seeds from which to establish new populations in historic fringed orchid habitats. A demographic monitoring system will be designed which will provide valuable life history information necessary to establish these new populations and make decisions on how to better manage existing populations.

Background

The western prairie fringed orchid, *Platanthera praeclara*, is a federally listed threatened species whose range occurs in the tall grass prairies of the central United States (U.S. Fish and Wildlife Service, 1989). Based on county records, a 60% decline in *P. praeclara* has been observed (Harrison 1989), which has primarily been caused by habitat loss and highly specific pollinator requirements. The drastic decline in population numbers poses a threat to the genetic variability among the populations. As genetic variability decreased, populations become less able to adapt to change which threatens their long term persistence in the face of environmental change and catastrophic events. The short term population fluctuation characteristic of the fringed orchids have made it difficult to determine whether individuals per population have been declining; however, the small numbers of individuals characteristic of the Iowa populations may lead to reduced genetic variation within individual populations (Pleasants and Klier 1995).

Recovery plans for threatened and endangered plants must take into account the population demography applied at the community and landscape levels. The fate of each population of the fringed orchid depends on its genetic, demographic, and environmental history dynamics. Widespread catastrophes and local environmental stochasticity can cause the extinction of entire populations at once because they affect all members of a populations. However, demographic and genetic stochasticity affects individuals so when population sizes are small there is an increased chance of the entire population becoming extinct (McEachern *et al* 1993). These small populations also may not attract enough pollinators which leads to reduced seed production and because sexual reproduction is necessary for long term persistence of the fringed orchid populations it is imperative that plans for population preservation by developed (Pleasants and Moe 1990).

Long term survival of metapopulations requires that population establishment rates equal or exceed extinction rates and that demographic processed and environmental events act on populations independently in order for some to be able to survive (McEachern *et al* 1993). Another important consideration is that the genetic diversity of the receptor site must not be lower than that of the source sit (Given 1994). A range of ten to fifty individuals is the recommended samples size; however, collection at the high end of the range is desirable due to the increased genetic diversity a large sample would provide (Guerrant 1992).

Proposed Work

The only previous genetic study of the fringed orchids (Pleasants and Klier 1995) utilized allozyme analysis. Very little genetic diversity was detected between and among populations of the eastern and western prairie fringed orchids; however these results are typical for protein analysis. Research, which I am conducting for my master's thesis, examines diversity among and between populations of the fringed orchids at the nucleotide base level. Two different methods of analysis are being utilized, randomly amplified polymorphic DNA (RAPD) and microsatellite nuclear DNA.

Microsatellites are short tandem repeats of nucleotide bases which are found interspersed throughout the genomes of prokaryotes and eukaryotes. They have a higher rate of mutation that other types of markers; and therefore, tend to be better indicators polymorphic alleles in organisms. I am developing my own primers which are specific for the western prairie fringed orchids, and will most likely work for the rest of the fringed orchids.

RAPD analysis is a fast and inexpensive method of comparison which I will also be using; however, the results are not as accurate and are difficult to reproduce. Random primers are utilized and those which produce polymorphic bands are chosen for analysis.

Through use of the polymerase chain reaction (PCR), RAPD primers and microsatellite primers are used to amplify small segments of DNA from the western prairie fringed orchid. For RAPD analysis, these fragments will be run electrophoretically on an agarose gel and stained with ethidium bromide in order to examine diversity between and within populations. Microsatellite nuclear DNA markers will be analyzed using an automated sequencer and run through a series of algorithms to determine relatedness and variation within and between populations.

Based on the results of the genetic analysis of the potential donor populations, recommendations will be made as to which populations(s) will be used to supply the founder individuals for the recipient sites. Native prairies which have historically contained the fringed orchids and still contain viable habitat for the plants will be used as locations for new populations establishment. Hand pollination of the orchids at the determined donor sites will be used to induce seed production which will be used to hand seed the receptor sites. This technique has produced successful results in Illinois populations (personal communication with M. Bowles). Also, soil testing of the receptor sites for the associated soil fungus, *Rhyzoctonis ap.*, necessary for seed germination would increase the chance for successful colonization of the receptor sites.

Demographic monitoring of existing populations is imperative in order to ensure the persistence of existing populations and establishment of new populations. Rather than a basic census to follow population sizes, the reproductive viability of the populations must be followed over time. This includes, but is not limited to, counts of the number of flowers per inflorescence, fruit sets per flower, and examination of pollinia to determine if the plant has been visited by a pollinator. Individuals in all life stages should also be marked in order to accurately determine the longevity of one plant. The number of leaves per plant should also be recorded to determine their role in long term survival of individuals. Following Menges guidelines, monitoring programs should also include visual estimations of vegetative cover, light and soil-water potential at the transect level and descriptions of dominant vegetation. Studies by Pleasants and Moe revealed no correlation between stand height and frequency of pollination; however, Cathrall recorded a positive correlation between plant height and fruit sets. This factor should be further examined in order to determine its importance in pollination of individuals. This would be a continuous monitoring project which could be repeated by others in future years.

Potential Sites

Due to concern about maintaining local ecotypes, donor sites will be paired with receptor sites in the same ecoregion. Studies of the historical locations of the eastern prairie fringed orchid are incomplete, therefore herbarium specimens must be examined before reintroduction projects involving the eastern species can be undertaken.

Western Prairie Fringed Orchid

donor sites:	Sheeder Prairie, Guthrie County
	Steele Prairie (larger tract), Cherokee County
receptor sites:	Cayler Prairie, Dickinson County
	Anderson Prairie, Emmet County
	Steele Prairie (smaller tract), Cherokee County

Preliminary Budget

first year - $5,000
- investigator's salary - $3,000 ($8.50/hr - 350hrs)
- travel (gas, lodging, etc) - $750
- lab and field supplies - $1,000
- misc. Expenses - $250

second year - $5,000
- investigator's salary - $3,000 ($8.50/hr - 350hrs)
- travel (gas, lodging, etc) - $750
- field supplies (i.e. protective enclosures to prevent grazing, etc.) - $1,000
- misc. expenses - $250

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POPULATION AND HABITAT VIABILITY ASSESSMENT (PHVA) FOR THE WESTERN PRAIRIE FRINGED ORCHID (*Platanthera praeclara*)

Eugene Mahoney State Park
Ashland, Nebraska
27-30 April 1997

Final Report

Appendix III.
IUCN Policy Guidelines

DRAFT GUIDELINES FOR RE-INTRODUCTIONS

Introduction

These policy guidelines have been drafted by the Re-introduction Specialist Group of the IUCN's Species Survival Commission (Guidelines for determining procedures for disposal of species confiscated in trade are being developed separately by IUCN for CITES.) in response to the increasing occurrence of reintroduction projects world-wide, and consequently, to the growing need for specific policy guidelines to help ensure that the re-introductions achieve their intended conservation benefit, and do not cause adverse side-effects of greater impact. Although the IUCN developed a Position Statement on the Translocation of Living Organisms in 1987, more detailed guidelines were felt to be essential in providing more comprehensive coverage of the various factors involved in re-introduction exercises.

These guidelines are intended to act as a guide for procedures useful to re-introduction programmes and do not represent an inflexible code of conduct. Many of the points are more relevant to re-introductions using captive-bred individuals than to translocations of wild species. Others are especially relevant to globally endangered species with limited numbers of founders. Each re-introduction proposal should be rigorously reviewed on its individual merits. On the whole, it should be noted that re-introduction is a very lengthy and complex process.

This document is very general, and worded so that it covers the full range of plant and animal taxa. It will be regularly revised. Handbooks for re-introducing individual groups of animals and plants will be developed in future.

1. Definition of Terms

a. "Re-introduction":

An attempt to establish a species (The taxonomic unit referred to throughout the document is species: it may be a lower taxonomic unit [e.g. sub-species or race] as long as it can be unambiguously defined.) in an area which was once part of its historical range, but from which it has become extinct (CITES criterion of "extinct": species not definitely located in the wild during the past 50 years. of conspecifics.). ("Re-establishment" is a synonym, but implies that the re-introduction has been successful) .

b. "Translocation":

Deliberate and mediated movement of wild individuals or populations from one part of their range to another.

c. "Re-inforcement/Supplementation":
Addition of individuals to an existing population.

d. "Conservation/Benign Introductions":

An attempt to establish a species, for the purpose of conservation, outside its recorded distribution but within an appropriate habitat and eco-geographical area.

2. Aims and Objectives of the Re-Introduction

a. Aims:

A re-introduction should aim to establish a viable, free-ranging population in the wild, of a species or subspecies which was formerly globally or locally extinct (extirpated). In some circumstances, a re-introduction may have to be made into an area which is fenced or otherwise delimited, but it should be within the species' former natural habitat and range, and require minimal long-term management.

b. Objectives:

The objectives of a re-introduction will include: to enhance the long-term survival of a species; to re-establish a keystone species (in the ecological or cultural sense) in an ecosystem; to maintain natural biodiversity; to provide long-term economic benefits to the local and/or national economy; to promote conservation awareness; or a combination of these.

Re-introductions or translocations of species for short-term, sporting or commercial purposes - where there is no intention to establish a viable population - are a different issue, beyond the scope of these guidelines. These include fishing an(i hunting activities.

3. Multidisciplinary Approach

A re-introduction requires a multidisciplinary approach involving a team of persons drawn from a variety of backgrounds. They may include persons from: governmental natural resource management agencies; non-governmental organisations; funding bodies; universities; veterinary institutions; zoos (and private animal breeders) and/or botanic gardens, with a full range of suitable expertise. Team leaders should be responsible for coordination between the various bodies and provision should be made for publicity and public education about the project.

4. Pre-Project Activities

a. Biological

(i) Feasibility study and background research

- An assessment should be made of the taxonomic status of individuals to be re-introduced.

They must be of the same subspecies as those which were extirpated, unless adequate numbers are not available. An investigation of historical information about the loss and fate of individuals from the re-introduction area, as well as molecular genetic studies, should be undertaken in case of doubt. A study of genetic variation within and between populations of this and related taxa can also be helpful. Special care is needed when the population has long been extinct.

- Detailed studies should be made of the status and biology of wild populations (if they exist) to determine the species' critical needs; for animals, this would include descriptions of habitat preferences, intraspecific variation and adaptations to local ecological conditions, social behaviour, group composition, home range size, shelter and food requirements, foraging and feeding behaviour, predators and diseases. For plants it would include biotic and abiotic habitat requirements, dispersal mechanisms, reproductive biology, symbiotic relationships (e.g. with mycorrhizae, pollinators), insect pests and diseases. Overall, a firm knowledge of the natural history of the species in question is crucial to the entire re-introduction scheme.

- The build-up of the released population should be modelled under various sets of conditions, in order to specify the optimal number and composition of individuals to be released per year and the numbers of years necessary to promote establishment of a viable population.

- A Population and Habitat Viability Analysis will aid in identifying significant environmental and population variables and assessing their potential interactions, which would guide long-term population management.

(ii) Previous Re-introductions

- Thorough research into previous re-introductions of the same or similar species and wide-ranging contacts with persons having relevant expertise should be conducted prior to and while developing re-introduction protocol.

(iii) Choice of release site

- Site should be within the historic range of species and for an initial re-inforcement or re-introduction have very few, or no, remnant wild individuals (to prevent disease spread, social disruption and introduction of alien genes). A conservation/ benign introduction should be undertaken only as a last resort when no opportunities for re-introduction into the original site or range exist.

- The re-introduction area should have assured, long-term protection (whether formal or otherwise).

(iv) Evaluation of re-introduction site

- Availability of suitable habitat: re-introductions should only take place where the habitat and landscape requirements of the species are satisfied, and likely to be sustained for the for-seeable future. The possibility of natural habitat change since extirpation must be considered. The area should have sufficient carrying capacity to sustain growth of the

re-introduced population and support a viable (self-sustaining) population in the long run.

- Identification and elimination of previous causes of decline: could include disease; over-hunting; over-collection; pollution; poisoning; competition with or predation by introduced species; habitat loss; adverse effects of earlier research or management programmes; competition with domestic livestock, which may be seasonal.

- Where the release site has undergone substantial degradation caused by human activity, a habitat restoration programme should be initiated before the reintroduction is carried out.

(v) Availability of suitable release stock

- Release stock should be ideally closely-related genetically to the original native stock.

- If captive or artificially propagated stock is to be used, it must be from a population which has been soundly managed both demographically and genetically, according to the principles of contemporary conservation biology.

- Re-introductions should not be carried out merely because captive stocks exist, nor should they be a means of disposing of surplus stock.

- Removal of individuals for re-introduction must not endanger the captive stock population or the wild source population. Stock must be guaranteed available on a regular and predictable basis, meeting specifications of the project protocol.

- Prospective release stock must be subjected to a thorough veterinary screening process before shipment from original source. Any animals found to be infected or which test positive for selected pathogens must be removed from the consignment, and the uninfected, negative remainder must be placed in strict quarantine for a suitable period before retest. If clear after retesting, the animals may be placed for shipment.

- Since infection with serious disease can be acquired during shipment, especially if this is intercontinental, great care must be taken to minimize this risk.

- Stock must meet all health regulations prescribed by the veterinary authorities of the recipient country and adequate provisions must be made for quarantine if necessary.

- Individuals should only be removed from a wild population after the effects of translocation on the donor population have been assessed, and after it is guaranteed that these effects will not be negative.

b. Socio-Economic and Legal Activities

- Re-introductions are generally long-term projects that require the commitment of
- long-term financial and political support.

- Socio-economic studies should be made to assess costs and benefits of the e-introduction programme to local human populations.

- A thorough assessment of attitudes of local people to the proposed project is necessary to ensure long term protection of the re-introduced population, especially if the cause of species' decline was due to human factors (e.g. over-hunting, over-collection, loss of habitat). The programme should be fully understood, accepted and supported by local communities.

- Where the security of the re-introduced population is at risk from human activities, measures should be taken to minirnise these in the re-introduction area. If these measures are inadequate, the re-introduction should be abandoned or alternative release areas sought.

- The policy of the country to re-introductions and to the species concerned should be assessed. This might include checking existing national and international legislation and regulations, and provision of new measures as necessary. Re-introduction must take place with the full permission and involvement of all relevant government agencies of the recipient or host country. This is particularly important in re-introductions in border areas, or involving more than one state.

- If the species poses potential risk to life or property, these risks should be minimised and adequate provision made for compensation where necessary; where all other solutions fail, removal or destruction of the released individual should be considered.

In the case of migratory/mobile species, provisions should be made for crossing of international/state boundaries.

5. Planning. Preparation and Release Stages

- Construction of a multidisciplinary team with access to expert technical advice for all phases of the programme.

- Approval of all relevant government agencies and land owners, and coordination with national and international conservation organizations.

- Development of transport plans for delivery of stock to the country and site of re-introduction, with special emphasis on ways to minimize stress on the individuals during transport.

- Identification of short-and long-term success indicators and prediction of programme duration, in context of agreed aims and objectives.

- Securing adequate funding for all programme phases.

- Design of pre- and post- release monitoring programme so that each re-introduction is a carefully designed experiment, with the capability to test methodology with scientifically collected data.

- Appropriate health and genetic screening of release stock. Health screening of closely related species in re-introduction area.

- If release stock is wild-caught, care must be taken to ensure that: a) the stock is free from infectious or contagious pathogens and parasites before shipment and b) the stock will not be exposed to vectors of disease agents which may be present at the release site (and absent at the source site) and to which it may have no acquired immunity.

- If vaccination prior to release, against local endemic or epidemic diseases of wild stock or domestic livestock at the release site, is deemed appropriate, this must be carried out during the "Preparation Stage" so as to allow sufficient time for the development of the required immunity.

- Appropriate veterinary or horticultural measures to ensure health of released stock throughout programme. This is to include adequate quarantine arrangements, especially where founder stock travels far or crosses international boundaries to release site.

- Determination of release strategy (acclimatization of release stock to release area; behavioural training - including hunting and feeding; group composition, number, release patterns and techniques; timing).

- Establishment of policies on interventions (see below).

- Development of conservation education for long-term support; professional training of individuals involved in long-term programme; public relations through the mass media and in local community; involvement where possible of local people in the programme.

- The welfare of animals for release is of paramount concern through all these stages.

6. Post-Release Activities

- Post release monitoring of all (or sample of) individuals. This most vital aspect may be by direct (e.g. tagging, telemetry) or indirect (e.g. spoor, informants) methods as suitable.

- Demographic, ecological and behavioural studies of released stock.

- Study of processes of long-term adaptation by individuals and the population.

- Collection and investigation of rnortalities.

- Interventions (e.g. supplemental feeding; veterinary aid; horticultural aid) when necessary.

- Decisions for revision, rescheduling, or discontinuation of programme where necessary.

- Habitat protection or restoration to continue where necessary.

- Continuing public relations activities, including education and mass media coverage.

- Evaluation of cost-effectiveness and success of re- introduction techniques.

- Regular publicationsin scientific and popular literature.

Approved by the 27th Meeting
of the IUCN Council

IUCN POLICY STATEMENT ON RESEARCH INVOLVING SPECIES AT RISK OF EXTINCTION

PROLOGUE

IUCN holds that all research on or affecting a threatened species carries a moral responsibility for the preservation or enhancement of the survival of that species. Conservation of the research resource is clearly in the interest of the researchers.

IUCN recognizes that the taking and trading of specimens of threatened species are covered by international agreements and are normally included in national legislation which provides authorized exemptions for the purpose of scientific research.

Basic and applied research is critically needed on many aspects of the biology of animal and plant species at risk of extinction (e g. those listed by IUCN as Vulnerable, Rare, Endangered, or indeterminate) to provide knowledge vital to their conservation.

Other scientific interests may involve the use of threatened species in a wide variety of studies. Taking into account the importance of many kinds of research, as well as potential threats such species could be subject to in such activities, IUCN, after careful consideration, adopts the following statements as policy.

POLICY

IUCN encourages basic and applied research on threatened species that contributes to the likelihood of survival of those species.

When a choice is available among captive-bred or propagated, wild-caught or taken, or free-living stock for research not detrimental to the survival of a threatened species, IUCN recommends the option contributing most positively to sustaining wild populations of the species.

IUCN recommends that research programmes on threatened species that do not directly contribute to conservation of the species should acknowledge an obligation to the species by devoting monetary or other substantial resources to their conservation, preferably to sustaining populations in the natural environment.

Whether animals involved are captive-bred, wild-caught, or free living, or whether plants

involved are propagated, taken from the wild, or in their natural habitat, IUCN opposes research that directly or indirectly impairs the survival of threatened species and urges that such research not be undertaken.

PROTOCOLS

In this context IUCN urges researchers to accept a personal obligation to satisfy themselves that the processes by which research specimens are acquired (including transportation) conform scrupulously to procedures and regulations adopted under international legal agreements. Further, researchers should adopt applicable professional standards for humane treatment of animal specimens, including their capture and use in research.

IUCN urges that any research on threatened species be conducted in conformity with all applicable laws, regulations and veterinary professional standards governing animal acquisition, health and welfare, and with all applicable agricultural and genetic resource laws and regulations governing acquisition, transport, and management of plants.