Eastern Prairie Fringed Orchid
(Platanthera leucophaea)

5-Year Review:
Summary and Evaluation

U.S. Fish and Wildlife Service
Chicago Illinois Field Office
Chicago, Illinois

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5-YEAR REVIEW
Eastern Prairie Fringed Orchid (*Platanthera leucophaea*)

1.0 GENERAL INFORMATION

1.1 Reviewers

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1.2 Methodology used to complete the review:

The U.S. Fish and Wildlife Service (Service) conducts status reviews of species on the List of Endangered and Threatened Wildlife and Plants (50 CFR 17.12) as required by section 4(c)(2)(A) of the Endangered Species Act (Act) (16 U.S.C. 1531 *et seq.*). The Service provided notice of this status review via the Federal Register (77 FR 38762) on June 29, 2012, requesting new information on the eastern prairie fringed orchid (*Platanthera leucophaea*) that may have a bearing on its classification as threatened. In addition, we applied information from a population viability assessment based on a compilation of data collected from across the species range (USFWS 2014, unpub. data), and relied upon this information most heavily. The Service's Chicago, Illinois Field Office conducted the review. We received comments from the Ohio Field Office (Jennifer Finfera) and the Missouri Field Office (Paul McKenzie).

1.3 Background:

1.3.1 FR Notice citation announcing initiation of this review: 72 FR 38762 (June 29, 2012) Endangered and Threatened Wildlife and Plants; 5 –Year Status Reviews of Seven Listed Species.

1.3.2 Listing history

Original Listing
FR notice: 54 FR 39857-39863
Date listed: Thursday, September 28, 1989
Entity listed: Species
Classification: Threatened

1.3.3 Associated rulemakings: Not applicable

1.3.4 Review History:

The eastern prairie fringed-orchid was included in a cursory 5-year review conducted for all species listed before 1991 (56 FR 56882). Since that time, a 5 year review was completed in 2010.

1.3.5 Species' Recovery Priority Number at start of 5-year review: 8 – This priority number indicates a species with a moderate degree of threat and high potential for recovery.

1.3.6 Recovery Plan

Name of plan: Eastern Prairie Fringed Orchid (*Platanthera leucophaea* [Nuttall] Lindley) Recovery Plan
Date issued: September 9, 1999
Dates of previous revisions, if applicable: The recovery plan has not been revised.

2.0 REVIEW ANALYSIS

2.1 Application of the 1996 Distinct Population Segment (DPS) policy

2.1.1 Is the species under review a vertebrate?

No

2.2 Recovery Criteria

2.2.1 Does the species have a final, approved recovery plan containing objective, measurable criteria?

Yes

2.2.2 Adequacy of recovery criteria.

2.2.2.1 Do the recovery criteria reflect the best available and most up-to date information on the biology of the species and its habitat?

Yes

2.2.2.2 Are all of the 5 listing factors that are relevant to the species addressed in the recovery criteria (and is there no new information to consider regarding existing or new threats)?

Yes, see below for explanation and there is new information.

2.2.3 List the recovery criteria as they appear in the recovery plan, and discuss how each criterion has or has not been met, citing information.

The Eastern Prairie Fringed Orchid Recovery Plan (USFWS 1999) contains the following delisting criteria:

1. **Twenty-two populations are distributed across plant communities and physiographic regions within the historic range of the species.** Currently 98 populations exist throughout the range of the species; however, the populations are not distributed as specified in the recovery plan (i.e., by state and physiographic region). The highly viable populations must occur in eight physiographic regions to achieve this criterion. Currently, nine highly viable populations occur in four of the eight physiographic regions required in the Recovery Plan. Therefore, Criterion 1 has not been met.

   Criterion 1 addresses issues associated with Factor A – present or threatened destruction, modification or curtailment of its habitat or range.

2. **Each of these 22 populations is highly viable. A highly viable population typically has more than 50 flowering plants; a population trend that is stable or increasing over a monitoring period of 5 years; available habitat of at least 50 hectares (125 acres) in size; assurances of ongoing management to reduce impacts from drainage, invasive non-native plant species or woody vegetation encroachment; and protection through long-term conservation easements, legal dedication as nature preserves, or other means.** Currently only nine populations are highly viable; Criterion 2 has not been met.

   Criterion 2 addresses issues associated with all listing factors: Factor A - present or threatened destruction, modification or curtailment of its habitat or range; Factor B - overutilization for commercial, recreational, scientific, or educational purposes; Factor C - disease or predation; Factor D - inadequacy of existing regulatory mechanisms; and Factor E - other natural or manmade factors affecting its continued existence.

The recovery criteria listed above were based upon the population viability index in the Eastern Prairie Fringed Orchid Recovery Plan (USFWS 1999) and Bowles *et al.* (1992). The viability index relies upon several types of measurements to fully assess the viability of a population. These variables include population size, potential habitat availability, the need for management, whether the habitat has long-term protection, and whether the population trend is increasing. See Appendix 1 for a brief description of each of these variables that indicate the viability of an eastern prairie fringed orchid population. The population viability index (Table 1) is based on these biological and habitat variables. For each variable, a ranking from 0-3 is assigned and the sum of all variable rankings is divided by the number of variables (e.g. five variables would yield a maximum sum of 15) to produce an index ranging from 0-1. Where no data is available, the variable is not included in the viability assessment. Populations with an index greater than 0.75 have high viability, populations with an index between 0.50 – 0.75 have moderate viability, and populations with an index less than 0.50 have low viability. Under this index, population viability is determined using a number of factors, and gives a more accurate indication of a population's viability or likelihood of persisting.

Table 1. Determination of Population Viability Index (PVI). Low population viability ≤.50 PVI, moderate viability ≥.50-.75 PVI, and high population viability >0.75 PVI.

	<---------- Range of Values ---------->			
Variable	(0)	(1)	(2)	(3)
I. Population size[1]	<10 (very small)	10-<25 (small)	25-<50 (medium)	>50 (large)
II. Population trend[2]	absent	decreasing	stable	increasing
III. Population reproduction frequency[3]	<33%	33-≤50%	50-≤67%	>67%
IV. Habitat size[4]	<1ha (very small) (<2.5 acres)	1<25 ha (small) (2.5<62.5 acres)	25<50 ha (medium) (62.5<125 acres)	>50 ha (large) (>125 acres)
V. Habitat condition and successional stage[5]	very heavily disturbed/early-successional	heavily disturbed / early-successional	moderately disturbed /mid-successional	lightly disturbed / late-successional
VI. Protection status [6]	none	informal	formal	legal
VII. Management condition[7]	severe	moderate	low	none

[1] Size derived from mean annual census data of flowering plants.
[2] Trend based on partial correlation (excluding rainfall and temperature) of annual population size with time. Decreasing = significant negative correlations, increasing = significant positive correlation.
[3] Percent frequency of years in which 10 percent or more of the flowering plants within a population produce seed.
[4] Area of potential habitat within an area occupied by orchids.
[5] Based on disturbance and successional stage.

[6] Function of ownership and deed restrictions. None = private ownership with no protection, informal = private ownership with informal protection agreements but without legally binding protection, formal = private or public ownership with formal but not legal protection, legal = private or public ownership with legally binding protection.
[7] Degree of management needed due to habitat degradation from fire suppression and woody plant succession, non-native plant species invasion, hydrology alteration, and other land use impacts.

Achieving Criterion 1:

The recovery plan (USFWS 1999) provides additional detail showing how the 22 highly viable populations should be distributed across plant communities, physiographic regions, and states (Table 2).

Table 2: The number of highly viable populations needed for recovery in each plant community, physiographic region, and state along with the number of eastern prairie fringed orchid populations and the viability of each population across the species' range from population viability analyses (PVA) conducted in 1999, 2007, and 2014 (USFWS 1999).

COMMUNITY / Physiographic Region / State	Number of high viability populations needed for recovery	High Viability 1999, 2007, 2014	Moderate Viability 1999, 2007, 2014	Low Viability 1999, 2007, 2014	Extirpated 1999, 2007, 2014
PRAIRIE					
Kansan Till					
Iowa *	2		1, 2	2	
Missouri				2	
Wisconsinan drift					
Illinois	4	1, 2, 3	10, 8, 7	7, 12, 15	3, 1
Indiana				1	
Wisconsin	3	2, 1, 1	5, 6, 6	2, 5	
Rock River Hill Country					
Illinois		1	1		
Lake Erie Lake Plain	2				
Michigan			1, 1	2	
Ohio		2	4, 2, 6	1, 3, 2	
Lake Huron Lake Plain	3				
Michigan		2, 2	3, 2	3, 7, 8	1
Lake Michigan Lake Plain	2				
Illinois			1, 1, 2	1, 3, 3	1
Wisconsin		1, 1, 1	1	1, 1, 2	
Southern MI / Northern IN Drift Plains					
Michigan				2	
SEDGE MEADOW	3				

Wisconsinan drift-unglaciated					
Illinois*		1	2, 2, 3	3,3	1
Ohio		1	3, 4, 1	1, 4	
Wisconsin		1, 1	1	2, 2, 2	
Unglaciated					
Iowa			1, 1	1	
Lake Erie Lake Plane					
Ohio			1		
MINEROTROPHIC/ SPHAGNUM PEATLAND	3				
Maine			1, 1, 1		
Michigan			3, 1, 1	2, 2	1
Wisconsin		1, 1	1		
Totals	22	6, 11, 9	37, 29, 32	16, 36, 55	7, 1

* One population exists (is extant) in this state, physiographic region, and community but was unable to be included in this table due to the lack of sufficient data to determine viability.

Shading denotes a physiographic region not originally identified in the Recovery Plan

As outlined in the Recovery Plan (USFWS 1999), in the Kansan Till physiographic region of Iowa two highly viable populations are required where currently none exist; four highly viable populations are required in prairies within the Wisconsinan Drift physiographic region of Illinois where currently three exist; three highly viable populations are required in prairies within the Wisconsinan Drift physiographic region of Wisconsin where currently one exists; two highly viable populations are required in prairies within the Lake Erie Lake Plain physiographic region of either Michigan or Ohio where currently none exist; three highly viable populations are required in prairies within the Lake Huron lake plain physiographic region of Michigan where currently none exist; two highly viable populations are required in prairies within the Lake Michigan lake plain physiographic region of either Illinois or Wisconsin where currently one exists in Wisconsin. According to the Recovery Plan (1999) three highly viable populations are required in a sedge meadow community and in either the physiographic region of Wisconsinan drift-unglaciated or unglaciated, and from any of the following states: Illinois, Iowa, Ohio, or Wisconsin, currently two highly viable populations exist within these criteria. Three highly viable populations are required in minerotrophic/sphagnum peatland in Maine, Michigan, or Wisconsin where currently one exists in Wisconsin. No physiographic region has reached the required number of highly viable populations needed for recovery.

Since the Recovery Plan (USFWS 1999) was written, previously unknown populations have been discovered and one population was created from restored habitat. These populations do not fall within the designated physiographic regions described in the Recovery Plan (USFWS 1999). These physiographic regions are included in Table 2 within the red shaded boxes. Within these physiographic regions, there is one population that is highly viable, one that is moderately viable

and two that are of low viability. All populations are considered when determining whether recovery criteria have been met.

Achieving Criterion 2:
Detailed analysis of the biological and habitat variables (Appendix 1) used in the population viability analysis from 1999, 2007, and 2014 provides additional insight into the species status and recovery needs. A summary of the variables and an update of how each variable has an influence on the viability of populations across the species range are provided below.

Population size: A population size of more than 50 flowering plants would be more resistant to effects of chance genetic, demographic, or environmental events that could most easily lead to population extirpation. Therefore, populations with 50 or more flowering plants rank the highest in this category. Typically, in an orchid population, some (or most) plants are not flowering, so counting the flowering plants underestimates the total population size. In 1999, 13 eastern prairie fringed orchid populations had 50 plants or more (USFWS 1999). In 2007, 15 of the 59 original populations (known in 1999) had on average 50 or more flowering plants (Bell 2008). The total number of extant populations in 2007 with an average of 50 plants or more was 18 of the 76 extant populations or an increase of five populations from 1999. In 2014, 23 of the 98 extant populations have an average of 50 or more flowering plants.

Population trend: Populations that have an increasing trend rank the highest in this category. In 1999, this variable was not used to determine population viability because trend data was not yet available. In 2007, 12 of the 59 original populations (known in 1999) were identified as increasing (Bell 2008). The total number of extant populations in 2007 that were identified as increasing was 17 of 76 populations. In 2014, only 9 of the 98 extant populations show an increasing population trend which may be the result of a range wide drought in 2012 which is believed to have significantly decreased population numbers in 2012 and 2013 range wide.

Habitat size: Populations in habitats larger than 50 hectares (125 acres) will support large numbers of plants and therefore rank the highest in this category. Based on the management condition of a site, eastern prairie fringed orchids may only occupy a portion of the potential habitat if some of the habitat has been encroached by invasive species. Habitat size is based on the potential habitat at the site. In 1999, nine eastern prairie fringed orchid populations occurred within 125 acres or greater of habitat (USFWS 1999). In 2007 there were 11 populations that occupied habitat of 125 acres or greater (Bell 2008). This increase was due to the discovery of previously unknown extant populations. In 2014, 13 of the 98 known populations persist in habitats of 125 acres or greater.

Habitat condition and successional stage: The successional stage, or "natural quality" (White 1978) of a site, is an indicator of past or current disturbance impacts to vegetation. Highly viable eastern prairie fringed orchid populations occur in late-successional habitat in high quality natural areas that are free of invasive species.

Fire and other management techniques that mimic natural disturbance may be required to control or eliminate invasive species and to maintain stable late-successional vegetation. Orchid populations may be more stable in late-successional (i.e., high quality natural area free of invasive species) plant communities. In 1999, 23 eastern prairie fringed orchid populations were in habitats exhibiting a late-successional stage (USFWS 1999). In 2007, 17 of the 76 extant populations were supported in habitats of a late successional stage (Bell 2008). The decrease of six populations (from 1999 to 2007) that are in habitats of a late-successional stage illustrates the continual threat of invasive species in eastern prairie fringed orchid habitat, and the need for continual management. In 2014, of the 98 extant populations, 22 are considered in a late-successional stage. The increase from 17 (2007) to 22 (2014) populations in late successional stage may be due to successful habitat management.

Protection status: Populations protected under binding legal conservation easements, including dedication under some state nature preserve acts, have the highest level of protection and therefore are ranked the highest in this category. Public land that is preserved in perpetuity and/or provides for regulatory protection would be examples of land that would also be ranked highest in this category. In 1999, 18 of 59 eastern prairie fringed orchid populations had binding legal protection (USFWS 1999). The total number of extant populations in 2007 with binding legal protection was 23 of 76 populations indicating an increase of five legally protected populations since 1999 (Bell 2008). This increase was due to the discovery of a previously unknown extant population, legal dedication of sites since 1999, and successful reintroduction in legally designated sites. The total number of populations with legal binding protection in 2014 is 43.

Management need: Eastern prairie fringed orchid populations are susceptible to woody vegetation encroachment and invasion by aggressive non-native plant species. In addition, management may be needed to mimic a lost function such as hydrology. This variable is assigned a value based on the degree of management needed to maintain the plant community (USFWS 1999; Bowles *et al.* 1992). In assessing this variable, the Service evaluates whether needed management is likely to continue in the future, and not just whether the site currently is free of management needs. Populations without a management need, rank the highest. In 1999, 12 eastern prairie fringed orchid populations had no management need (USFWS 1999). In 2007, 12 of the 59 original populations (known in 1999) had no management need (Bell 2008). The total number of extant populations as of 2007 with no management need was 13 of 76 populations (USFWS 1999, Bell 2008). In 2014 the total number of populations without management need is 4 out of 98 populations.

2.3 Updated Information and Current Species Status

2.3.1 Biology and Habitat

2.3.1.1 New information on species' biology and life history:

Dr. Timothy Bell (The Morton Arboretum / Chicago State University) analyzed demographic monitoring data that was collected annually, during the peak flowering season in June or July and again when fruits were mature in September (Bell *et al.* 2015). The demographic dataset used in this study represents 36 Illinois eastern prairie fringed orchid populations and consists of 5943 permanently marked plants from 1998-2012. Monitoring data included presence/absence, height, leaf number, length of the longest leaf, number of flowers, number of hand-pollinated flowers, number of fruits, herbivory severity, and whether the plant location was burned since last growing season.

Population Matrix Modeling:

Dr. Bell found that based on the demographic transition matrix pooled over all populations and years, the eastern prairie fringed orchid (in Illinois) has an increasing population growth rate (λ = 1.1391) (Bell *et al.* 2015). The mean generation time (or average age for first reproduction of a cohort) for the eastern prairie fringed orchid is 3.95 years (Bell *et al.* 2015). However, only about 30% of plants live more than one year after entering the demographic dataset (Bell *et al.* 2015). Survival and flowering probability differ among plant stages (juvenile, flowering, and vegetative (vegetative plants are nonflowering plants that flowered at least once before)) and generally increase with plant size. Most plants stay in a respective stage for about 1 year, however some plants (< 1%) can repeatedly flower for up to 6 years (Bell *et al.* 2015).

Additional research results (Bell *et al.* 2015) include:

- Most plants live only 1 year, or they live one year after they are found at a site and also included in the demographic dataset. Specifically, 98% of plants live five years or less and 2% live 6 to 12 years.
- Most plants stay in a respective stage only for 1 year. Flowering and vegetative (nonflowering plants that flowered at least once before) plants can stay in that stage for up to 6 years.
- Most plants are never dormant (94%). Of those that are dormant (6%), 67% are dormant for a year, 20% are dormant for 2 years, 9% are dormant for 3 years, 2% are dormant for 4 years, 0.5% are dormant for 5 years.
- Survival is the highest in relatively large plants.
- Large juveniles are most likely to flower the following year.
- Plants that are hand pollinated have a slightly higher pod production rate ($p = 0.42$).
- There is estimated to be 4,500 seeds in each seed capsule.
- If all of the offspring of one plant were found, we would expect to find 22 juveniles.
- The proportion of survival for this orchid by herbivory level:
    - Five categories were determined (none, slight leaf damage, slight flower damage, severe flower damage, entire inflorescence destroyed).
    - Plants with no herbivory had lower survival than those with slight flower damage.
    - Herbivory does not appear to present a problem based on overall survival information.
    - The effects of herbivory on flowering the following year does not appear to have a clear effect.

- Prescribed burning increases population survival and does not affect the number of flowering plants or pods.
- Populations with low management needs have a higher mean survival rate.

Projected Population Matrix Modeling:

Dr. Bell created a matrix which models population dynamics that we would expect if we found all of the plants in the field, based on what we know from tagged plants. The matrix model was run for 100 years with estimations of population persistence given different management needs. Populations with low management needs persisted for a long time (100 years), but populations with severe management needs persisted for only 50 years (Bell *et al.* 2015).

In evaluating the effects of management activities on survival, fecundity and population viability, logistic regression indicated that survival was significantly higher for plants in areas with prescribed burn ($R_N^2 = 0.105$, $P < 0.001$) and with low management needs ($R_N^2 = 0.011$, $P < 0.001$) (Bell *et al.* 2015) (Bell *et al.* 2015). Survival was lower for plants with severe herbivory damage ($R_N^2 = 0.004$, P 0.004), but was not affected by hand pollination ($R_N^2 = 0.001$, $P = 0.100$) (Bell *et al.* 2015). However, fecundity was significantly higher for plants in areas with prescribed burn ($F = 4.023$, $P < 0.001$) and hand pollinated plants ($t = 2.034$, $P = 0.042$), and lower for plants with severe herbivory damage ($F = 93.555$, $P = 0.045$) (Bell *et al*. 2015). Population viability analysis using a demographic matrix model indicated that population growth rates (λ) were significantly higher for plants in areas with prescribed burn ($\lambda_{burn} = 1.78$, $\lambda_{unburn} = 1.22$), normal rainfall ($\lambda_{drought} = 0.75$, $\lambda_{normal} = 1.27$, $\lambda_{wet} = 0.82$), and with crossing between populations ($\lambda_{outcrossing\ btw\ pops} = 1.79$, $\lambda_{outcrossing\ within\ pops} = 1.13$, $\lambda_{selfing} = 0.91$), but did not differ among management needs or herbivory levels (Bell *et al.* 2015). These results may suggest that prescribed burn and hand pollination among populations can improve eastern prairie fringed orchid population viability (Bell *et al.* 2015).

Other factors affecting population viability were also modeled (Bell *et al.* 2015). In normal weather, with burning, and crossing (via hand pollination) between populations the population growth rates are projected to increase. Weather cannot be controlled, but we can control burning and crossing. Dr. Bell's analysis suggests that these practices (prescribed burning and crossing between populations) could be used to enhance population growth rates.

Soil Mycorrhizae:

Dr. Lawrence Zettler (Illinois College) has pioneered research between soil mycorrhizae and the symbiotic relationship of *P. leucophaea* seed germination. It is assumed that strains of mycorrhizae utilized by most of the Illinois eastern prairie fringed orchid populations are assignable to the anamorphic genus *Ceratorhiza* (Note: mycologists worldwide now prefer to use the teleomorphic name, *Ceratobasidium* instead of the anamorphic name *Ceratorhiza*, and the recent literature now reflects this change. We have retained the anamorphic name in this document for consistency). Members of this genus are likely able to utilize nitrate which could be available in soil after frequent burning. Thus, it is conceivable that the eastern prairie fringed orchid receives a nitrogen supply, in part, from the fungus in burned areas. Previous work identified two *Ceratorhiza* species that typically associate with North American prairie orchids.

In vitro, *Ceratorhiza* induced rapid growth of *Platanthera* seeds when placed in direct contact with the fungus. Eastern prairie fringed orchids, like other terrestrial orchids, are generally regarded as 'parasites' of mycorrhizal fungi at early germination stages given that the fungus is consumed as a carbon source (=mycotrophy) with little or no benefit to the fungus at the onset of the symbiosis. Once the orchid seedling initiates photosynthesis, eastern prairie fringed orchids and many other orchids are thought to retain their mycotrophic capability, supplementing photosynthesis depending on its carbon needs. Although much research is still needed, prairie ecosystems and terrestrial orchids therein (namely *Platanthera*) appear to harbor strains of *Ceratorhiza* on a persistent basis, but the exact whereabouts of a given strain of *Ceratorhiza* in a particular area of prairie is difficult to assess without seed baiting techniques (see Zettler and Piskin, 2011, and commentary below). The presence of *Ceratorhiza* in roots of mature eastern prairie fringed orchids as well as very young seedlings (protocorms) suggests that this orchid utilizes this fungus to a large extent. Consequently, the presence/absence of *Ceratorhiza* is believed to be a major limiting factor in the persistence of this orchid.

One way to determine if and where *Ceratorhiza* is present at a site is to place *P. leucophaea* seed baits (nylon mesh seed packets) along transects at sites to find which area of a habitat the *Ceratorhiza* actually grows in. If the seeds germinated, then *Ceratorhiza* was present. As a management tool, seed baits could be used to determine exact locations of this fungus in an area of a prairie before sowing the eastern prairie fringed orchid seeds within that habitat. This would ensure knowing exactly where in the landscape the *Ceratorhiza* fungus is located. However, the seed bait process can take up to 2 years. It is important to note that the absence of fungus via seed baiting does not necessarily mean that the fungus is not present at the site. It only identifies exact areas of a site where no fungus occurs, with other areas of the site possibly supporting the fungus.

There are 75 strains of mycorrhizae identified from *Platanthera leucophaea.* Seventeen isolates are cryopreserved at the University of Alberta in Canada and 18 more are being added, however *Ceratorhiza* does not always store well.

In 2012, two strains of mycorrhizal fungi were isolated from *P. leucophaea* from one McHenry County, Illinois population. Unlike most previous collections spanning populations in Illinois, Michigan, and Wisconsin, these fungi were identified as *Tulasnella (Epulorhiza)* strains. One was identified as an *Epulorhiza* strain, the other a *Ceratorhiza* strain. These two isolates were subsequently deposited into the University of Alberta Microfungus Collection and Herbarium (UAMH) culture collection in Canada. *Ceratorahiza* appears to be the primary genus *P. leucophaea* utilizes for its mycotrophic needs throughout its life, and preserving strains assignable to this genus is critical. Unfortunately, *Ceratorhiza* strains are more difficult to cryopreserve compared to *Epulorhiza.*

In 2014, Dr. Zettler collected mycorrhizal fungus samples from 3 eastern prairie fringed orchid sites in Illinois and one site in Michigan. Dr. Larry Zettler and two of his students (Illinois College) had grown out the fungus from these sites and had samples of the fungus from each site to give back to the appropriate personnel from those sites for an experimental inoculation process. The mycorrhizae were not used at a site more than 50 miles from where it originated.

In 2014 transects were marked in suitable orchid habitat at 3 existing eastern prairie fringed orchid sites in Illinois. The transects were marked at 1.5 meter intervals where a shallow hole was dug. Every other hole was then inoculated with the mycorrhizae. Every other 1.5 meter hole was left with no mycorrhizae as a control. The mycorrhiza was left to grow all season and in the fall, eastern prairie fringed orchid seed was placed in each hole. If successful, within 2 years (at these Illinois sites) we may see the first strap leaf plants of an eastern prairie fringed orchid plant instead of waiting 6 to 9 years to see the first blooming plant after the site is sown with eastern prairie fringed orchid seed.

2.3.1.2 Abundance, population trends, demographic features, or demographic trends:

In 2008, a rangewide population viability assessment for the eastern prairie fringed orchid was completed based on data collected across the range from 1999 to 2007 (Bell 2008). The results from this research (Table 2) indicated that there were 76 known extant populations or 17 more populations than in 1999. Additionally, there were 11 highly viable populations, an increase of five since 1999. Other differences from the 1999 population viability assessment included a decrease from 37 to 29 populations with moderate viability and an increase from 16 to 36 populations with low viability.

In 2014 a third rangewide population viability assessment for the eastern fringed prairie orchid was conducted based on data collected from 2008-2014. This analysis (Table 2) shows there are currently 98 extant populations rangewide, 22 more than reported in 2007. The number of populations exhibiting high viability decreased from 11 (2007) to 9 (2014). The number of populations exhibiting moderate viability increased from 29 (2007) to 32 (2014). The number of populations exhibiting low viability also increased from 36 (2007) to 55 (2014). The increase by 22 populations from 2007 to 2014 is due to the discovery of new populations, introduction efforts, and in some cases, the division of a population that was once considered 1 population (in 2007) but now is considered two or more separate populations (in 2014). New population division accounts for six new populations in Wisconsin. Rangewide we have 9 new populations that are considered to be of low viability and 10 that are considered to be of moderate viability. In Illinois there are four new introduced populations since 2007.

In Illinois, demographic monitoring and hand pollination have been used as recovery strategies and tools to guide efforts to augment extant populations. Data collected in the Illinois orchid program is used to track the status of individual populations. This data indicates fluctuations in population size from year to year, with the seemingly more drastic shifts in population size occurring following a drought (Keibler et al 1993; Keibler 1994, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2003; TNC 2007). These findings agree with earlier research (Bowles 1983) that moisture levels are an important factor in the promotion of eastern prairie fringed orchid growth. Drought in 2012 likely influenced population sizes in 2012 and also influenced population sizes in 2013, as it is believed that this species exhibits a lag effect in response to stress (drought).

Research conducted by Vitt (2001and 2003) found that pollinating 30% of eastern prairie fringed orchid flowers maximizes both reproduction and survival of the species. This research supports the methodology used in Illinois recognizing that this may be impractical to continue in

perpetuity without considerable volunteer assistance. In addition, the data collected in the Illinois orchid program is used to track seed production from natural pollination and hand pollination. These data provide a guide as to how populations are being augmented and where seed may be collected and disbursed for reintroduction in Illinois.

2.3.1.3 Genetics, genetic variation, or trends in genetic variation:

Increased concern over potentially elevated levels of inbreeding at small populations in Illinois, where hand pollination only occurs within that population, led to a discussion by participants at the 2012 Illinois Eastern Prairie Fringed Orchid Researcher / Landowner Meeting on future genetic management practices at Illinois eastern prairie fringed orchid populations. Specifically, the group questioned whether cross pollination should occur between populations? Past genetic studies provided some background to guide this decision. A white paper was written which summarized the past genetic work (up until 2012) on the orchid across its range (Appendix 4). This information was presented to both the Illinois Eastern Prairie Fringed Orchid Researcher / Landowner Team and the Illinois Eastern Prairie Fringed Orchid Volunteer Team (responsible for monitoring and hand pollination within their sites) who all work to recover the species.

The Illinois Eastern Prairie Fringed Orchid Researcher / Landowner Team decided that cross pollination between populations was likely to enhance recovery of the species. Specific criteria for cross pollination between populations were established as described below:

1) Focus on crossing small (<50 plants) populations.
2) Focus on sites within 50 miles from each other.
3) Overall ecological health and quality of the site should be taken into consideration along with whether there will be long term commitment to the management of these sites.
3) Cross as closely as possible within similar plant communities (prairie or sedge meadow).
4) The site should have had blooming orchid plants within at least the last 10 years.
5) Do not augment populations with high deer populations (even if a robust EPFO population is established, each blooming orchid would have to be caged which may not be a good use of time and energy.)
6) Crosses and seed dispersal should contribute to recovery.
    a. Follow the Recovery Plan in trying to cross-pollinate populations where, if they produce larger populations, they would contribute to recovery in the appropriate plant community and physiographic regions needed for recovery from Illinois.
        i. Eastern prairie fringed orchid sites in underrepresented physiographic regions may receive priority.
    b. The highest priority sites would be those that have the best likelihood to increase the population viability from moderate to high. Using the Population Viability Assessment (FWS 2014), these would be moderately viable sites with small eastern prairie fringed orchid populations. These sites would receive the highest priority in regards to being the first to receive donated pollen or seed.

Recent Genetic Research:

Low genetic diversity and high levels of inbreeding are a potential threat to *P. leucophaea* populations because these two factors could lead to inbreeding depression. A recent study (Paul *et al.* 2013) investigated whether the reduction in eastern prairie fringed orchid population sizes in Canada has resulted in low genetic diversity and increased inbreeding. The effect of hybridization with *P. psycodes* was also investigated. Paul *et al.* (2013) found low genetic diversity and high levels of inbreeding in all populations, including those that hybridize. Paul *et al.* (2013) suggests genetic monitoring of *P. leucophaea* every 5 years to assist in management of the species.

Current Genetic Research:

Claire Ellwanger (MS student at Northwestern Illinois University / The Chicago Botanic Garden / FWS intern) is conducting a current range-wide genetic analysis of the eastern prairie fringed orchid.

During the summer of 2015 fresh leaf samples were collected from 32 historically large extant populations rangewide (IA, IL, IN, MO, ME, MI, OH, WI). Of these 32 populations, seven populations had preserved DNA from 1998 (18 years ago) which will allow for genetic comparison of these seven populations which have undergone different genetic management techniques over time. Over two-hundred and fifty herbarium specimens from historic locations of extinct and extant eastern prairie fringed orchid populations have been identified and contacted in an effort to include samples of their specimens in this study.

Genetic variation will be assessed using microsatellite primers developed for *Platanthera praeclara* (Ross *et al.* 2013), which were tested on the eastern prairie fringed orchid in the fall of 2015. Populations will be compared for genetic diversity (allelic richness, alleles per loci, expected heterozygosity (He), inbreeding (Fis), and genetic differences between populations (Fst). These results will be compared to *P. praeclara* (Ross *et al.* (in prep)), another hawkmoth pollinated species which has not seen as dramatic of a decline as *P. leucophaea*. Comparing results of these two studies will provide a contrast of the diversity and genetic structure of both species and help inform and guide recovery and conservation planning for the eastern prairie fringed orchid.

Contemporary range-wide analysis may identify conservation priority populations (high diversity) as well as those at risk due to elevated inbreeding or low diversity. Inclusion of herbarium specimens allows for a complete analysis of genetic variation across the historic range of the species that would otherwise require extrapolation from present day genetic patterns. Knowledge of historic and present day range-wide genetic variation is useful to determine if there has been a loss of diversity with range contraction, identify the migration route for this species during the last ice age, and perhaps predict potential migration patterns or future range shifts. Results from this research will evaluate whether recovery actions are achieving the goal of reducing inbreeding and maintaining genetic diversity. Final results from this study are pending.

2.3.1.3 Taxonomic classification or changes in nomenclature:

There has been no new information regarding taxonomic classification or nomenclature since the 1991 Review.

2.3.1.4 Spatial distribution, trends in spatial distribution, or historic range:

The eastern prairie fringed orchid's distribution has not changed appreciably since 1991.

2.3.1.6 Habitat or ecosystem conditions:

Habitat loss and degradation continue to threaten the eastern prairie fringed orchid. Ongoing management is needed at all orchid sites for woody and invasive species control. When resources are available the Service has funded management at some eastern prairie fringed orchid sites, but reliance on conservation organizations, local and state government agencies, as well as volunteer commitment, to implement management activities are the primary means to successful eastern prairie fringed orchid site management. In 2007 at the Ottawa National Wildlife Refuge in Ohio, habitat management led to the discovery of a previously unknown *P. leucophaea* population totaling 127 plants (Huffman 2009).

2.3.1.7 Other:

Three species of hawkmoths (*Eumorpha pandorus*, *Eumorpha achemon*, and *Sphinx eremitis)* have been verified as eastern prairie fringed orchid pollinators (Cuthrell 1994, Crosson *et al.* 1999, Cuthrell *et al.* 1999, Pollack 2009). More research is needed on the hawkmoths' distribution, population levels, management needs, reproduction, and effects from threats (e.g. pesticide use).

2.3.2 Five-Factor Analysis

2.3.2.1 Present or threatened destruction, modification or curtailment of its habitat or range:

Most eastern prairie fringed orchid populations have been lost through conversion of habitat to cropland and pasture. Drainage and development pose threats to this species' habitat. In addition, late-successional (i.e., high quality natural areas free of invasive species) prairie remnants supporting this species require management to reduce cover of woody vegetation. Fire and other management techniques that mimic natural disturbance may be required to control or eliminate invasive species and to maintain stable late successional vegetation. Most sites within the species range need continual management. In addition, if past actions have destroyed some ecosystem function (i.e., natural drainage) then management may be needed to mimic the lost function. Lack of appropriate natural areas management threatens populations regardless of their legal protection status (USFWS 1989).

2.3.2.2 Overutilization for commercial, recreational, scientific, or educational purposes:

Native terrestrial orchids are rarely grown from seed. Adult plants are often sought for scientific and commercial purposes or, for gardens and therefore are susceptible to collection. Smaller populations of eastern prairie fringed orchids can be negatively

impacted by collecting. Due to high human population densities in some parts of the range of the eastern prairie fringed orchid, it can be subject to collection pressures. In the past, populations of eastern prairie fringed orchids in Michigan and Illinois have been impacted by removal of plants (USFWS 1989).

2.3.2.3 Disease or predation:
Although no threats were identified under this listing factor when the species was listed (USFWS 1989), an increase in deer populations in portions of the species range (e.g. Illinois) has resulted in an increased impact from herbivory of eastern prairie fringed orchid flowers which reduces or eliminates the plants ability to reproduce. In Illinois, deer cages are provided by the Fish and Wildlife Service if the volunteer stewards believe their blooming eastern prairie fringed orchid plants would benefit from deer caging. In recent years, destruction of adult flowering plants later in the season (after seed production and before seed dispersal) from voles has been documented at many Illinois populations. Efforts to cage for voles has provided limited success.

2.3.2.4 Inadequacy of existing regulatory mechanisms:
Protection of threatened plants on privately-owned lands is extremely limited in most states throughout the eastern prairie fringed orchid's range, leaving those populations vulnerable to habitat destruction and extirpation (USFWS 1989). Currently and range wide, 43 of 98 existing populations, have full legal land protection (USFWS 2014).

2.3.2.5 Other natural or manmade factors affecting its continued existence:
The eastern prairie fringed orchid's dependence upon hawkmoths for pollination makes it vulnerable to changes in these insect populations. The status of most hawkmoth species is poorly known. Pollinator populations may be adversely affected by pesticides and loss of habitat (USFWS 1989).

Climate change will be a particular challenge for endangered, threatened and other at-risk species because the interaction of additional stressors associated with climate change and current stressors may push them beyond their ability to survive (Easterling and Karl 2000). In addition, populations of some species that are near the southern end of the range may be at particular risk (IPCC 2014). While there is uncertainty about the exact nature and severity of climate change related impacts anticipated within the eastern prairie fringed orchid's range, a number of scientific studies project that there will be increased duration and intensity of heat waves in summer, higher levels of humidity and evaporation; changing patterns of precipitation with fewer rain events of greater intensity; increased frequency and more severe dry spells; and more flooding from heavy rains (Easterling and Karl 2000; Ebi and Meehl 2007; Hall and Stuntz 2007; IPCC 2014; NCA 2014). Research has suggested that climate change may also negatively impact pollinator species if the plants and their pollinators respond differently to climate change (NRC 2007; Earthwatch Institute 2006). These climatic changes may threaten the eastern prairie fringed orchid in a variety of direct and indirect ways. However, climate changes will likely affect phenological timing, availability of suitable habitat, inter-specific relationships with pollinators and mycorrhizae associates, and threats from invasive species. Specific predictions of vulnerability or ability to shift ranges to suitable habitat in response to climate change based on

life-history traits are frequently found to be species specific, and not widely applicable (Angert *et al.* 2011). In an effort to predict the potential range shift of the eastern prairie fringed orchid under climate change, Dr. Pati Vitt (2007) used modeling to predict the climate in Illinois in 2095. Dr. Vitt's (2007) research suggests that the predicted range of the eastern prairie fringed orchid will be concentrated northwest of its current range (Vitt 2007). The current areas of concentration for this species are around the Great Lakes. In the future, Michigan and Canada may be the best locations for this species considering climate change (Vitt 2007). The range is predicted to shift out of the Midwest and up towards the northeast (Vitt 2007). Close monitoring of *P. leucophaea* populations will help detect the species response to climate change and allow for consideration of management options.

2.4 Synthesis

Overall, 39 more populations exist now than were known since the issuance of the recovery plan (USFWS 1999) and 22 more populations have been identified since the completion of the last population viability assessment in 2007. The increase in the number of populations is due in part to the discovery of previously unidentified, but naturally occurring populations. Long overdue habitat management led to another discovery of a previously unidentified population. The hand pollination of plants has ensured an adequate supply of seed for introduction into suitable habitat which in one case is responsible for a new population which is highly viable. Three more populations are ranked highly viable than were identified in the 1999 population viability assessment. However, habitat loss and degradation continue to threaten the eastern prairie fringed orchid. Many of the eastern prairie fringed orchid populations across the species' range have severe to moderate management needs and continue to be threatened by invasive species, woody vegetation encroachment, or changes in hydrology and development. Population trends for *Plantanthera leucophaea* indicate that population fluctuations are variable across the species range and may be influenced by climatic conditions, most notably precipitation.

Although progress has been made in protecting eastern prairie fringed orchid populations from invasive species encroachment, most populations are still threatened by invasive species and need to be managed on a continual basis with the best management practices available. Progress towards achieving the delisting criteria continues. Under Criterion 1, nine of the required twenty-two viable populations are distributed across plant communities and physiographic regions within the historic range of the species, but no plant community or physiographic region has met the recovery criterion. Under Criterion 2, twenty-two highly viable populations are required; currently only nine populations are highly viable. The five-factor threats analysis demonstrates that threats to EPFO populations are still occurring (i.e., destruction, modification or curtailment of its habitat, overutilization, predation by deer, inadequate regulatory mechanisms on non-Federal land, and natural or manmade factors affecting its continued existence). This species may become endangered in the foreseeable future throughout all or a significant portion of its range, and therefore, continues to meet the definition of threatened.

3.0 RESULTS

3.1 Recommended Classification:
No change is needed

3.2 New Recovery Priority Number: Not applicable

Brief Rationale: Recovery Criterion 1 states that twenty-two populations are distributed across plant communities and physiographic regions within the historic range of the species. Although twenty two additional populations have been identified since the 2007 population viability assessment, the numbers of populations needed within the plant communities or physiographic regions as described in the Recovery Plan have not been met. Criterion 2 states that each of these 22 populations must be highly viable. The total number of populations considered highly viable has decreased from 11 in 2007 to 9 in 2014; therefore criterion 2 has not been met. Habitat loss and degradation continue to threaten the eastern prairie fringed orchid. In addition, deer herbivory to flowering plants appears to be increasing across the species range. Though more populations exist, their threats have not diminished; therefore, no change in the classification (threatened) or recovery priority number (8) is suggested.

3.3 Listing and Reclassification Priority Number: Not applicable

4.0 RECOMMENDATIONS FOR FUTURE ACTIONS

The highest priority recovery actions for the eastern prairie fringed orchid are acquiring legal protection of habitat, and managing habitat. Protecting habitat through legal designation is recovery action 1 and identified as a priority 1 action (i.e., an action that must be taken to prevent extinction or to prevent the species from declining irreversibly in the foreseeable future) (USFWS 1999). In most states, the highest available form of legal protection consists of conservation easements under state nature preserve acts (Pearsall 1984). Because only 43 of the 98 (44%) extant eastern prairie fringed orchid populations have legal land protection status, protection under state nature preserve acts should be pursued for the remaining populations. For states that do not have active nature preserve acts (e.g., Michigan), other forms of conservation easements that can be held by private organizations should be sought. Another option available to private land-owners is conveyance of property rights to public or private conservation agencies that will provide legal protection and management.

Recovery action 2 addresses managing habitat (USFWS 1999). Because sites supporting orchid populations may require varying degrees of active management to maintain or enhance orchid populations, habitat management was identified in the species recovery plan as a priority 1 action. Only 4 of the 98 extant sites are considered without management need, a decrease from 13 in 2007. Currently, only 22 of the 98 known sites are in a late-successional stage. Management techniques needed may include prescribed burns, or brush and invasive species removal depending on the site condition. While habitat is being managed at many eastern prairie fringed orchid sites across the species range, habitat management is an ongoing activity that will have varying degrees of need based on the level of woody species encroachment and invasion by non-native plant species.

Recovery action 3 (increasing the size and number of populations) needs to be implemented continuously. As discussed above, through the removal of encroaching woody vegetation, eastern prairie fringed orchid habitat may be increased which, in turn, may lead to population expansion. The number of pollinator visits to small orchid populations may be a limiting factor for seed production at a particular site. Hand-pollination should be used where natural pollination is believed to be infrequent or absent in order to maximize seed production. Hand-pollination and seed dispersal appear to provide cost effective methods for augmenting existing populations (action 3.1) and reintroducing or introducing new populations (action 3.2) in appropriate habitat that is legally protected (USFWS 1999).

Much has been learned about the eastern prairie fringed orchid since its listing and completion of the Federal recovery plan. However, there is still a need for greater understanding of the species life history requirements, specifically the species' pollinators and seed germination. Three species of hawkmoths (*Eumorpha pandorus*, *Eumorpha achemon*, and *Sphinx eremitis)* have been verified as eastern prairie fringed orchid pollinators (Cuthrell 1994, Crosson *et al.* 1999, Cuthrell *et al.* 1999, Pollack 2009). However, little is known about the hawkmoths' distribution, population levels,

management needs, or reproduction. Research to gain greater understanding of these aspects of the pollinators will assist in the recovery of the eastern prairie fringed orchid (USFWS 1999, action 5.2).

Research to date has determined that the mycorrhizal fungus *Ceratorhiza goodyerae-repentis* promotes the germination of eastern prairie fringed orchid seed (Zettler *et al.* 2005) and can sustain mature plants (Zettler *et al*. 2001). In addition, the fungus *C. pernacatena* has also been recovered from mature eastern prairie fringed orchids (Zettler *et al*. 2001), suggesting that the species may associate with both *C. goodyerae-repentis* and *C. pernacatena* when mature (Zettler *et al.* 2005). Further research is needed to determine the extent that eastern prairie fringed orchids require these fungal species throughout its range. In addition, research to determine if *C. goodyerae-repentis* can be used to inoculate seedlings, introduce these orchids into potential restoration sites, and propagate eastern prairie fringed orchids *ex situ* is needed (USFWS 1999, action 5.3).

The three population viability assessments cited in this review are based on field surveys conducted between 1990 and 1998, from 1999 to 2007, and from 2008-2014 (USFWS 1999, Bell 2008, USFWS 2014). The data collected in the population viability assessments provide an accurate and distinct update of the status of the eastern prairie fringed orchid across the range of the species and are integral in completing this review. Assessment of the progress toward recovery through updates to the population viability assessment rankings should be completed annually, as described under action 6.1 (USFWS 1999).

Research Needed

The need exists to determine if EPFO mycorrhizal fungi (*Ceratorhizal/Ceratobasidium)* spanning different prairie sites are genetically similar or not. The need exists to sequence the existing strains (molecular ITS sequencing) in storage so that we have more confidence in the kinds of strains we could be releasing into new existing sites. Acquiring this knowledge will be important considering climate change projections (i.e., selecting potential new EPFO sites higher in latitude for assisted migration purposes if deemed appropriate)

The need exists to determine why EPFO seems to utilize *Ceratorhiza (Ceratobsidium)* over *Epulorhiza (Tulasnella)* and whether EPFO's dependence on *Ceratorhiza* is linked to frequent burning. Most other orchids seem to harbor (need) more *Epulorhia* strains than *Ceratorhiza,* but the reverse seems true for the prairie fringed orchids, including the sister species *P. praeclara. Ceratorhiza* has been recovered repeatedly from different sites, and from different growth stages of the orchid ranging from protocorms, seedlings and mature plants.

As identified in the species recovery plan, action 5.2 identifies the need for research to address the status of eastern prairie fringed orchid pollinators (hawkmoths), their vulnerability to human impacts (widespread pesticide use), and their life history requirements (e.g., habitat patch size, larval host plants, adult nectar plants, etc.) (USFWS 1999). Research is also needed to gain a greater understanding of aspects of the eastern prairie fringed orchid pollinators' distribution, population levels, management needs, and reproduction.

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Expert consulted:
Dr. Timothy Bell, Professor of Botany at Chicago State University and Research Associate at the Morton Arboretum, provided population viability data (2007) for the eastern prairie fringed orchid across the species range.

U.S. FISH AND WILDLIFE SERVICE 5-YEAR REVIEW
Species: Eastern prairie fringed orchid (*Platanthera leucophaea*)

Current Classification: Threatened

Recommendation resulting from the 5-Year Review:

____ Downlist to Threatened
____ Uplist to Endangered
____ Delist
_X__ No change needed

Appropriate Recovery Priority Number: ___8___

Review Conducted By: Cathy Pollack and Claire Ellwanger

FIELD OFFICE APPROVAL:

Lead Field Supervisor, U.S. Fish and Wildlife Service

Approve _____ Date _____

REGIONAL OFFICE APPROVAL:

Lead Assistant Regional Director, Ecological Services, U.S. Fish and Wildlife Service, Midwest Region

Approve _____ Date _____

REGIONAL CONCURRENCE:

Regional Director, U.S. Fish and Wildlife Service, Northeast Region

Signature_____ Date _____

APPENDIX 1

Variables Used in Eastern Prairie Fringed Orchid Population Viability Index

Population size: Eastern prairie fringed orchid flowering population trend statistics are drawn from annual flowering plant census data. Flowering plant numbers are important measures of viability because populations appear to rely on seed production for their maintenance. Population size estimates can be based on mean annual flowering plant census data, with the recognition that numbers of plants and proportion of flowering plants will vary annually. Mean annual flowering plant census data is assigned a value and applied to the index as follows: 0 = fewer than 10 flowering plants; 1 = 10 to <25 flowering plants; 2 = 25 to <50 flowering plants, 3 = greater than 50 flowering plants (USFWS 1999; Bowles *et al.* 1992).

Population extirpation simulations indicate that populations with fewer than 10 plants are highly vulnerable to effects of chance genetic, demographic, or environmental events that could most easily lead to population extirpation and a population size of more than 50 plants would be more resistant to these factors (Bowles and Bell 1999). Because populations include flowering and non-flowering plants, flowering plant census data will underestimate actual population sizes.

Population trend: Estimates of the population trend indicate whether the population size is stable, increasing, or decreasing over time, after accounting for variations in population size that follow annual rainfall and temperature fluctuations. This variable is a correlation of annual population size with time and is assigned values and applied in the index as follows: 0 = plants absent; 1 = decreasing or significant negative correlations; 2 = stable or no significant correlation; 3 = increasing or significant positive correlation (USFWS 1999; Bowles *et al.* 1992).

Population reproduction frequency: Reproduction population trend statistics are drawn from annual flowering plant census data. The frequency of years in which flowering plants reproduce directly affects population persistence by regulating the potential for seedling establishment. The frequency of years in which 10% or more of the flowering plants within a population produce seed is the measure for this variable and is a in the index as follows: 0 = frequency less than 33%; 1 = frequency between 33-50%; 2 = frequency between 50-67%; 3 = frequency greater than 67% (USFWS 1999; Bowles *et al.* 1992). However, data is rarely available to apply to this variable, so it has not been applied in population viability assessments conducted to date on the eastern prairie fringed orchid.

Habitat size: Larger habitats will support higher numbers of orchids, and may provide greater opportunity for surviving changing environmental conditions since orchids may colonize suitable areas if current habitat becomes unsuitable. Larger habitats are also more likely to support greater levels of natural disturbances, such as from habitat-size restricted animals, and thus more patch disturbance for orchid seedling establishment and potential for orchid colonization. Chances of extirpation might be highest in habitats smaller than 1 hectare (2.5 acres). Pioneer cemeteries, which often support the smallest prairie remnants found in the range of the eastern prairie fringed orchid, are usually no more than 2 hectares (5 acres) in size. Habitats larger than 50 hectares (125 acres) will support large numbers of plants. Values assigned to habitat size in the population viability index are applied as follows: 0 = habitat less than 2.5 acres; 1 = habitat

2.5 to <62.5 acres; 2 = habitat 62.5 <125 acres; 3 = habitat greater than 125 acres (USFWS 1999; Bowles *et al.* 1992).

Habitat successional stage and condition: The successional stage, or "natural quality" (White 1978), is an indicator of past or current disturbance impacts to vegetation. Highly viable eastern prairie fringed orchid populations occur in late-successional habitat in high quality natural areas that are free of invasive species. Fire and other management techniques that mimic natural disturbance may be required to control or eliminate invasive species and to maintain stable late-successional vegetation. Early to mid-successional communities can contain large orchid populations. However, these habitats are successionally unstable and orchid populations are at risk unless management can sustain optimum conditions under which the high population levels originated (Sheviak 1990), which may conflict with management for more stable late-successional prairie vegetation (Bowles *et al*. 1992). Values assigned to this variable are based on the degree of disturbance (i.e. natural quality grade) or habitat successional stage and are as follows: 0 = very heavily disturbed (grade D) or early successional; 1 = heavily disturbed (grade C) or early successional; 2 = moderately disturbed (grade B) or mid-successional; 3 = lightly or undisturbed (grade A) or late-successional (USFWS 1999; Bowles *et al.* 1992).

Protection status: Protection status is a function of ownership and legal deed restrictions. Public or private tracts protected under legal conservation easements, including dedication under some state nature preserve acts, have the highest level of protection. Public land that is preserved in perpetuity and/or provides for regulatory protection would be examples of land that would be considered highly viable. Habitats in public ownership that are not legally protected may have formal protection status but can be subject to management or use that could conflict with orchid habitat maintenance. Private land not protected by legal conservation easements might have informal protection such as volunteer registry programs and landowner agreements, but long-term land use remains at the discretion of the landowner. This variable is assigned a value based on ownership and legal deed restrictions as follows: 0 = private ownership with no protection; 1 = private ownership with informal protection agreements but without legally binding protection; 2 = private or public ownership with formal but not legal protection; 3 = private or public ownership with legally binding protection (USFWS 1999; Bowles *et al.* 1992).

Management condition: The eastern prairie fringed orchid occurs in grass- and sedge dominated communities that require fire to prevent woody vegetation encroachment. Fire and other management techniques that mimic natural disturbance may be required to control or eliminate invasive species and to maintain stable late-successional vegetation. Most sites within the species range will need almost continual management. In addition, if past actions have destroyed some ecosystem functions, then management may be needed to mimic the lost function. For example, drainage and water table loss can directly impact orchid populations and can also accelerate invasion by woody plant species. Invasion by aggressive non-native plant species such as glossy buckthorn, reed canary grass, and purple loosestrife can also require corrective action. Moderate management needs are for threats that are not directly impacting orchid populations, such as invasion of early stages of woody or non-native plant species, or surrounding land use. This variable is assigned a value based on the degree of management needed to maintain the plant community as follows: 0 = severe; 1 = moderate; 2 = low; 3 = none (USFWS 1999; Bowles *et al.* 1992).

APPENDIX 2

2007 Population Viability Assessment

Community	Physiographic Region	Site name	State	Population size	Population trend	Habitat size	Protection status	Successional status	Management needs	PVI	Viability
Prairie	Kansan till	Garden Grove Prairie	IA	1	2	2	0	3	2	0.56	Moderate
Prairie	Kansan till	Williams Prairie	IA	0	0	2	3	2	2	0.50	**Extirpated**
Prairie	Kansan till	Muskrat Slough	IA	3	1	1	2	2	2	0.61	Moderate
Prairie	Lake Erie lake plain	Monroe Co. #1	MI	3	2	1	2	1	1	0.56	Moderate
Prairie	Lake Erie lake plain	Mallard Club Wildlife Area	OH	3	3	2	2	2	2	0.78	High
Prairie	Lake Erie lake plain	Maumee Bay	OH	1	1	1	2	2	2	0.50	Low
Prairie	Lake Erie lake plain	Metzger	OH	3	1	1	0	2	2	0.50	Low
Prairie	Lake Erie lake plain	Ottawa National Wildlife Refuge	OH	3	2	3	3	2	2	0.83	High
Prairie	Lake Erie lake plain	Pickerel Creek	OH	3	2	1	2	1	2	0.61	Moderate
Prairie	Lake Erie lake plain	Wightman's Grove	OH	2	3	1	2	1	3	0.67	Moderate
Prairie	Lake Erie lake plain	Yodonta Rd	OH	1	1	1	0	2	2	0.39	Low
Prairie	Lake Huron lake plain	St. Clair County #2	MI	0	1	1	2	2	2	0.44	**Extirpated**
Prairie	Lake Huron lake plain	Huron County #1	MI	3	3	3	2	3	3	0.94	High
Prairie	Lake Huron lake plain	Tuscola County #1	MI	3	2	3	2	3	3	0.89	High
Prairie	Lake Huron lake plain	Bay County #1	MI	1	1	1	1	1	1	0.33	Low
Prairie	Lake Huron lake plain	Bay County #2	MI	0	2	1	2	1	1	0.39	Low
Prairie	Lake Huron lake plain	Bay County #3	MI	2	2	1	0	1	1	0.39	Low
Prairie	Lake Huron lake plain	Saginaw County #1	MI	1	2	1	0	0	1	0.28	Low

Community	Physiographic Region	Site name	State	Population size	Population trend	Habitat size	Protection status	Successional status	Management needs	PVI	Viability
Prairie	Lake Huron lake plain	St. Clair County #1	MI	1	2	0	1	2	2	0.44	Low
Prairie	Lake Huron lake plain	Tuscola County #2	MI	2	1	1	0	1	1	0.33	Low
Prairie	Lake Huron lake plain	Tuscola County #3	MI	0	0	1	2	3	3	0.50	Low
Prairie	Lake Michigan lake plain	Miami Woods FP	IL	0	0	1	2	2	2	0.39	**Extirpated**
Prairie	Lake Michigan lake plain	Gensburg-Markham Prairie	IL	0	2	1	0	3	1	0.39	Low
Prairie	Lake Michigan lake plain	Paintbrush Prairie*	IL	0	1	1	3	1	2	0.44	Low
Prairie	Lake Michigan lake plain	Sundrop Prairie*	IL	0	2	1	3	1	2	0.50	Low
Prairie	Lake Michigan lake plain	Illinois Beach	IL	0	2	2	3	3	3	0.72	Moderate
Prairie	Lake Michigan lake plain	Chiwaukee Complex	WI	3	2	3	3	3	3	0.94	High
Prairie	Lake Michigan lake plain	Bain Station	WI	1	0	2	1	1	1	0.33	Low
Minerotrophic /Spahgnum Peatland	n/a	Crystal Bog	ME	1	2	1	2	3	3	0.67	Moderate
Minerotrophic /Spahgnum Peatland	n/a	Livingston County #1	MI	0	2	1	2	1	1	0.39	Extirpated
Minerotrophic /Spahgnum Peatland	n/a	St. Joseph County #1	MI	1	2	0	0	2	2	0.39	Low
Minerotrophic /Spahgnum Peatland	n/a	St. Joseph County #2	MI	0	0	1	1	2	2	0.33	Low

Community	Physiographic Region	Site name	State	Population size	Population trend	Habitat size	Protection status	Successional status	Management needs	PVI	Viability
Minerotrophic /Spahgnum Peatland	n/a	Washtenaw County #1	MI	0	2	1	3	2	2	0.56	Moderate
Minerotrophic /Spahgnum Peatland	n/a	Cedarburg	WI	3	2	2	2	3	2	0.78	High
Prairie	Wisconsinan drift	Burlington Prairie*	IL	0	2	0				0.11	**Extirpated/ Low**
Prairie	Wisconsinan drift	Churchill Prairie	IL	0	0	2	2	1	1	0.33	**Extirpated**
Prairie	Wisconsinan drift	Lincolnshire	IL	0	2	1	2	2	2	0.50	Low
Prairie	Wisconsinan drift	Wolf Road Prairie	IL	0	2	1	3	2	2	0.56	**Extirpated**
Prairie	Wisconsinan drift	Grant Creek	IL	0	3	2	3	3	3	0.78	High
Prairie	Wisconsinan drift	Munson Cemetery	IL	3	3	1	3	3	3	0.89	High
Prairie	Wisconsinan drift	Abbott Park	IL	1	1	1	1	1	1	0.33	Low
Prairie	Wisconsinan drift	Baxter	IL	1	3	1	1	1	1	0.44	Low
Prairie	Wisconsinan drift	Carpentersville	IL	0	2	1	0	1	1	0.28	Low
Prairie	Wisconsinan drift	Florsheim NP	IL	0	1	1	3	1	1	0.39	Low
Prairie	Wisconsinan drift	Hybernia NP	IL	0	2	1	3	1	1	0.44	Low
Prairie	Wisconsinan drift	Nippersink + DeRose-Glacial Park	IL	0	2	2	2	1	2	0.50	Low
Prairie	Wisconsinan drift	Rudd Farm	IL	0	3	1	0	1	1	0.33	Low
Prairie	Wisconsinan drift	Schiller Woods FP	IL	0	2	1	2	1	2	0.44	Low
Prairie	Wisconsinan drift	Somme Woods FP	IL	0	3	1	2	1	2	0.50	Low
Prairie	Wisconsinan drift	Swift Prairie	IL	0	3	1	2	1	0	0.39	Low
Prairie	Wisconsinan drift	Wayside Prairie	IL	0	2	1	2	1	1	0.39	Low

Community	Physiographic Region	Site name	State	Population size	Population trend	Habitat size	Protection status	Successional status	Management needs	PVI	Viability
Prairie	Wisconsinan drift	Loda Cemetery	IL	0	3	1	3	3	2	0.67	Moderate
Prairie	Wisconsinan drift	Lone Grove*	IL	1	2	1	2	2	2	0.56	Moderate
Prairie	Wisconsinan drift	Lyons Woods	IL	2	2	1	3	2	2	0.67	Moderate
Prairie	Wisconsinan drift	Middlefork Savanna	IL	0	2	1	3	3	3	0.67	Moderate
Prairie	Wisconsinan drift	Somme NP*	IL	0	2	1	3	2	2	0.56	Moderate
Prairie	Wisconsinan drift	W. Chicago Prairie	IL	0	3	2	2	2	2	0.61	Moderate
Prairie	Wisconsinan drift	Wadsworth Prairie	IL	3	2	3	3	1	1	0.72	Moderate
Prairie	Wisconsinan drift	Wrigley tract	IL	2	2	2	1	1	2	0.56	Moderate
Prairie	Wisconsinan drift	Rock1 (Koshkonong)	WI	3	3	3	2	2	3	0.89	High
Prairie	Wisconsinan drift	Taylor Creek Prairie	WI	0	0	0	0	2	2	0.22	Low
Prairie	Wisconsinan drift	White River Marsh	WI	0	2	1	2	2	2	0.50	Low
Prairie	Wisconsinan drift	Faville-Snapper	WI	1	2	2	3	3	2	0.72	Moderate
Prairie	Wisconsinan drift	Greene	WI	0	2	2	2	3	3	0.67	Moderate
Prairie	Wisconsinan drift	Newark Rd	WI	1	2	1	2	3	2	0.61	Moderate
Prairie	Wisconsinan drift	Oshkosh-Larsen	WI	1	2	1	2	2	3	0.61	Moderate
Prairie	Wisconsinan drift	Scuppernong	WI	1	1	2	3	2	1	0.56	Moderate
Prairie	Wisconsinan drift	Young	WI	0	2	2	3	2	1	0.56	Moderate
Sedge Meadow	Wisconsinan drift / unglaciated	Baldwin Marsh	IA	3	1	1	1	1	2	0.50	Low
Sedge Meadow	Wisconsinan drift / unglaciated	Harrison Benwell	IL	0	0	1	2	1	1	0.28	**Extirpated**

Community	Physiographic Region	Site name	State	Population size	Population trend	Habitat size	Protection status	Successional status	Management needs	PVI	Viability
Sedge Meadow	Wisconsinan drift / unglaciated	Hildy Prairie	IL	3	3	1	1	2	2	0.67	Moderate
Sedge Meadow	Wisconsinan drift / unglaciated	Long Grove	IL	1	3	1	3	2	2	0.67	Moderate
Sedge Meadow	Wisconsinan drift / unglaciated	Dayton (Medway)	OH	3	2	1	0	2	2	0.56	Moderate
Sedge Meadow	Wisconsinan drift / unglaciated	Killbuck SM (Cemetary Rd)	OH	1	2	3	2	2	2	0.67	Moderate
Sedge Meadow	Wisconsinan drift / unglaciated	Killbuck SM (Holmesville)	OH	1	2	3	2	2	2	0.67	Moderate
Sedge Meadow	Wisconsinan drift / unglaciated	Killbuck SM (State Rt 83 site)	OH	2	3	3	2	2	2	0.78	High
Sedge Meadow	Wisconsinan drift / unglaciated	Leadingham	OH	3	1	1	1	2	2	0.56	Moderate
Sedge Meadow	Wisconsinan drift / unglaciated	South River SM	VA	0	0	3	0	2	1	0.33	**Extirpated**
Sedge Meadow	Wisconsinan drift / unglaciated	Uihlein (Waukau)	WI	3	3	1	3	3	2	0.83	High
Sedge Meadow	Wisconsinan drift / unglaciated	Desplaines	WI	0	1	3	1	2	2	0.50	Low
Sedge Meadow	Wisconsinan drift / unglaciated	Pell Lake	WI	0	1	0	0	2	1	0.22	Low
Prairie	Illinoisan drift	Nachusa*	IL	0	3	1	3	2	2	0.61	Moderate
Sedge Meadow	Wisconsinan drift	Ascension Sedge Meadow	IL	0	1	1	2	2	2	0.44	Low
Sedge Meadow	Wisconsinan drift	Silver Lake	IL	0	0	1	1	2	2	0.33	Low

Community	Physiographic Region	Site name	State	Population size	Population trend	Habitat size	Protection status	Successional status	Management needs	PVI	Viability
Sedge Meadow	Wisconsinan drift	Standlee Fen/Slough Creek Sedge Meadow	IL	0	2	1	0	1	1	0.28	Low

* = Site reintroduced by scattering or broadcasting seeds.

Appendix 3

2014 Population Viability Assessment

Community	Physiographic Region	Site name	State	Population size	Population trend	Habitat size	Protection status	Successional status	Management needs	PVI	Viability
Prairie	Lake Michigan lake plain	Miami Woods FP	IL	0	0	1	2	1.5	1.5	0.33	Low
Prairie	Lake Michigan lake plain	Gensburg-Markham Prairie NP	IL	0	0	1	3	3	2	0.5	Low
Prairie	Lake Michigan lake plain	Paintbrush Prairie NP	IL	0	0	1	3	3	2	0.5	Low
Prairie	Lake Michigan lake plain	Sundrop Prairie NP	IL	0	2	1	3	2	2	0.56	Moderate
Prairie	Lake Michigan lake plain	Illinois Beach State Park	IL	0	2	2	3	3	2	0.67	Moderate
Prairie	Wisconsinan drift	Burlington Prairie	IL	0	0	0	?	?	?		Extirpated
Prairie	Wisconsinan drift	Churchill Prairie NP	IL	0	2	2	3	1	1	0.5	Low
Prairie	Wisconsinan drift	Wolf Road Prairie NP	IL	0	0	1	3	2	1	0.39	Low
Prairie	Wisconsinan drift	Grant Creek NP	IL	2	2	2	3	3	2	0.78	High
Prairie	Wisconsinan drift	Munson Cemetery NP	IL	3	1	1	3	3	1	0.67	Moderate
Prairie	Wisconsinan drift	Abbott Park	IL	0	1	1	1	1	1	0.28	Low

Community	Physiographic Region	Site name	State	Population size	Population trend	Habitat size	Protection status	Successional status	Management needs	PVI	Viability
Prairie	Wisconsinan drift	Dokum Mscoda NP (Baxter)	IL	1	1	1	3	3	1	0.56	Moderate
Prairie	Wisconsinan drift	Helm Road	IL	0	1	1	0	1	0	0.17	Low
Prairie	Wisconsinan drift	Florsheim NP	IL	0	0	1	3	2	2	0.44	Low
Prairie	Wisconsinan drift	Hybernia NP	IL	0	2	1	3	2	1	0.5	Low
Prairie	Wisconsinan drift	Nippersink + DeRose-Glacial Park	IL	0	1	1	2	2	1	0.39	Low
Prairie	Wisconsinan drift	Rudd Farm	IL	0	1	1	0	1	0	0.17	Low
Prairie	Wisconsinan drift	Schiller Woods FP	IL	0	3	1	2	1	2	0.5	Low
Prairie	Wisconsinan drift	Swift Prairie FP	IL	0	1	1	3	1	1	0.39	Low
Prairie	Wisconsinan drift	Wayside Prairie	IL	0	0	1	2	2	1	0.33	Low
Prairie	Wisconsinan drift	Loda Cemetery NP	IL	0	1	1	3	3	2	0.56	Moderate
Prairie	Wisconsinan drift	Lone Grove	IL	2	1	1	2	2	1	0.5	Low
Prairie	Wisconsinan drift	Lyons Prairie & Woods NP	IL	3	1	1	3	2	2	0.67	Moderate
Prairie	Wisconsinan drift	Middlefork Savanna NP	IL	0	1	1	3	3	2	0.56	Moderate

Community	Physiographic Region	Site name	State	Population size	Population trend	Habitat size	Protection status	Successional status	Management needs	PVI	Viability
Prairie	Wisconsinan drift	W. Chicago Prairie NP	IL	0	1	2	3	2	1	0.5	Low
Prairie	Wisconsinan drift	Wadsworth Prairie NP	IL	3	2	3	3	2	1	0.78	High
Prairie	Wisconsinan drift	Wrigley tract	IL	2	1	2	1	2	1	0.5	Low
Sedge Meadow	Wisconsinan drift / unglaciated	Hildy Prairie NP	IL	3	2	1	3	2	3	0.77	High
Sedge Meadow	Wisconsinan drift / unglaciated	Long Grove NP	IL	0	1	1	3	1	0	0.33	Low
Sedge Meadow	Wisconsinan drift	Ascension Sedge Meadow FP	IL	1	2	1	2	3	2	0.61	Moderate
Sedge Meadow	Wisconsinan drift	Silver Lake	IL	?	?	1	1	?	?	?	insufficient data to determine
Sedge Meadow	Wisconsinan drift / unglaciated	Harrison Benwell	IL	0	0	1	2	0	0	0.17	Low
Sedge Meadow	Wisconsinan drift-unglaciated	Queen Anne	IL	0	2	0	2	2	1	0.39	Low
Prairie	Wisconsinan drift	HUM RR Prairie	IL	0	1	0	2	3	2	0.44	Low
Sedge Meadow	Wisconsinan drift-unglaciated	Bystricky Prairie NP	IL	0	2	1	3	3	2	0.61	Moderate
Sedge Meadow	Wisconsinan drift-unglaciated	Bystricky Pr (Steadman Parcel)	IL	3	2	1	3	1	1	0.61	Moderate

Community	Physiographic Region	Site name	State	Population size	Population trend	Habitat size	Protection status	Successional status	Management needs	PVI	Viability
Prairie	Wisconsinan drift	Glenview Air Station Pr.	IL	1	3	2	2	2	2	0.67	Moderate
Sedge Meadow	Rock River Hill Country	Nachusa	IL	3	3	1	3	2	2	0.77	High
Prairie	Wisconsinan drift	Somme NP	IL	0	2	1	3	3	2	0.61	Moderate
Prairie	Wisconsinan drift	Somme Woods FP	IL	3	3	1	2	3	2	0.78	High
Prairie	Lake Huron lake plain	Bradleyville Road (Bradford Rd. Prairie)	MI	0	1	1	1	2	0	0.27	Low
Prairie	Lake Huron lake plain	Sebewaing Bay South	MI	3	1	3	1	2-3	1	0.61 - 0	Moderate
Prairie	Lake Huron lake plain	Essexville Prairie	MI	0	1	1	0	0	0	0.11	Low
Prairie	Lake Erie lake plain	Pointe Mouillee North	MI	0	1	1	0	0	0	0.11	Low
Prairie	Lake Huron lake plain	Harsens Island - Middle Channel Golf Course	MI	0	1	0	0	0	0	0.06	Low
Prairie	Lake Erie lake plain	Pointe Mouillee South	MI	3	1	1	1	0	0	0.33	Low
Minerotrophic/ Spahgnum Peatland	NA	George Reserve	MI	0	1	1	1	2	1	0.33	Low
Prairie	Lake Huron lake plain	Cotter Road North/Coryeon Point	MI	1	1	1	1	2	0	0.33	Low
Prairie	Lake Huron lake plain	Wildfowl Bay Prairie	MI	3	2	3	2	2-3	1-2	0.72 - 0	Moderate
Minerotrophic/ Spahgnum Peatland	NA	Little Cedar Lake	MI	1	2	1	1	3	2	0.56	Moderate
Prairie	Lake Huron lake plain	Dickinson Island	MI	0	1	1	0	2	0	0.22	Low

Community	Physiographic Region	Site name	State	Population size	Population trend	Habitat size	Protection status	Successional status	Management needs	PVI	Viability
Prairie	Lake Huron lake plain	Zilwaukee	MI	1	2	1	0	1	0	0.28	Low
Prairie	Southern Michigan / Northern Indiana drift plain (?)	Tamarack Lake	MI	1	2	1	0	3	2	0.5	Low
Prairie	Lake Huron lake plain	Clements Airport	MI	1	1	1	0	0	3	0.33	Low
Prairie	Lake Huron lake plain	Fish Point	MI	0	1?	1	1	2-3	1?	0.33 - 0	Low
Prairie	Southern Michigan / Northern Indiana drift plain (?)	Williams Lake	MI	0	?	0	1	2	2	0.33	Low
Minerotrophic/ Spahgnum Peatland (?)	NA	Dowagiac Creek - LaGrange Lake	MI	0	?	0	0	1	2	0.2	Low
Prairie	Lake Michigan lake plain	Bain Station	WI	0	0-1	1	0	2	1	0.27	Low
Minerotrophic/ Spahgnum Peatland	NA	Cedarburg	WI	3	2	3	3	3	1	0.83	High
Prairie	Lake Michigan lake plain	Chiwaukee - Barnes	WI	0	2	1	2	1	2	0.44	Low
Prairie	Lake Michigan lake plain	Chiwaukee - Lakeshore Drive	WI	0	2	2	2	2	2	0.55	Moderate
Prairie	Lake Michigan lake plain	Chiwaukee - South	WI	3	2	3	3	2	2	0.83	High

Community	Physiographic Region	Site name	State	Population size	Population trend	Habitat size	Protection status	Successional status	Management needs	PVI	Viability
Sedge Meadow	Wisconsinan drift-unglaciated	Des Plaines	WI	0	0-1	3	2	1	1	0.44	Low
Prairie	Wisconsinan drift	Faville-Snapper	WI	2	2	1	3	3	2	0.72	Moderate
Prairie	Wisconsinan drift	Greene	WI	0	2	1	3	2	2	0.55	Moderate
Prairie	Wisconsinan drift	Koshkonong (Rock1)	WI	3	2	3	2	3	2	0.83	High
Prairie	Wisconsinan drift	Newark Rd	WI	1	2	1	3	3	1	0.61	Moderate
Prairie	Wisconsinan drift	Oshkosh-Larsen - Allenville	WI	0	0-1	0	3	2	2	0.44	Low
Prairie	Wisconsinan drift	Oshkosh-Larsen - Breezewood	WI	0	1	0	3	2	2	0.44	Low
Prairie	Wisconsinan drift	Oshkosh-Larsen - Maxwell	WI	0	2	1	2	1	1	0.39	Low
Sedge Meadow	Wisconsinan drift-unglaciated	Pell Lake	WI	0	0 or 2	1	0	1	1	0.27	Low
Prairie	Wisconsinan drift	Scuppernong	WI	1	2	1	3	1	2	0.55	Moderate
Prairie	Wisconsinan drift	Snapper Prairie	WI	0	2	1	3	3	2	0.61	Moderate
Prairie	Wisconsinan drift	Taylor Creek Prairie	WI	0	2	1	0	1	0	0.22	Low
Sedge Meadow	Wisconsinan drift-unglaciated	Uihlein (Waukau)	WI	3	2	3	3	3	2	0.88	High

Community	Physiographic Region	Site name	State	Population size	Population trend	Habitat size	Protection status	Successional status	Management needs	PVI	Viability
Prairie	Wisconsinan drift	White River Marsh	WI	0	2	3	3	2	2	0.67	Moderate
Prairie	Wisconsinan drift	Young	WI	0	2	1	3	2	1	0.5	Low
Prairie	Wisconsinan drift (?)	Lowe Prairie	IN	0	0	1	3	2	2	0.44	Low
Prairie	Lake Erie lake plain	Mallard Club Wildlife Area	OH	3	1	2	2	2	1	0.61	Moderate
Prairie	Lake Erie lake plain	Maumee Bay	OH	3	1	1	2	2	1	0.56	Moderate
Prairie	Lake Erie lake plain	Metzger	OH	1	2	1	0	2	2	0.44	Low
Prairie	Lake Erie lake plain	Ottawa NWR - Crane Creek	OH	3	2	1	3	1	2	0.67	Moderate
Prairie	Lake Erie lake plain	Ottawa NWR - Cedar Point	OH	0	3	3	3	1	2	0.67	Moderate
Sedge Meadow	Lake Erie lake plain	Ottawa NWR - Cedar Young	OH	2	3	2	3	1	2	0.72	Moderate
Prairie	Lake Erie lake plain	Pickerel Creek	OH	3	2	1	2	2	1	0.61	Moderate
Prairie	Lake Erie lake plain	Wightman's Grove	OH	3	2	1	2	1	3	0.67	Moderate
Prairie	Lake Erie lake plain	Yodonta Rd	OH	0	1	0	0	2	1	0.22	Low
Sedge Meadow	Wisconsinan drift/unglaciated	Dayton (Medway)	OH	3	1	1	0	2	1	0.44	Low
Sedge Meadow	Wisconsinan drift/unglaciated	Killbuck SM (Cemetary Rd)	OH	0	1	1	2	2	2	0.44	Low
Sedge Meadow	Wisconsinan drift/unglaciated	Killbuck SM (Holmesville)	OH	1	2	1	2	2	1	0.5	Low

Community	Physiographic Region	Site name	State	Population size	Population trend	Habitat size	Protection status	Successional status	Management needs	PVI	Viability
Sedge Meadow	Wisconsinan drift/unglaciated	Killbuck SM (State Rt 83 site)	OH	1	1	1	2	2	1	0.44	Low
Sedge Meadow	Wisconsinan drift/unglaciated	Leadingham	OH	3	3	2	1	1	1	0.61	Moderate
Prairie	Kansan Till	North Evans	MO	1	1	0	1	3	3	0.5	Low
Prairie	Kansan Till	Cottonwood Swale	MO	2	1	1	1	2	2	0.5	Low
Menerotropic/ Sphagnum Peatland	NA	Cedarburg Bog	ME	1	2	1	3	2	1	0.56	Moderate
Sedge Meadow	Wisconsinan drift/unglaciated	Baldwin Marsh	IA	3	1	3	2	2	1	0.66	Moderate
Prairie	Kansan Till	Muskrat Slough	IA	1	1	2	2	2	0	0.44	Low
Prairie	Kansan Till	Williams Prairie	IA	0	?	2	3	?	?	?	insufficient data to determine
Prairie	Kansan Till	Garden Grove Prairie	IA	0	0	1	0	2	1	0.22	Low

APPENDIX 4

Consideration of the Potential Benefits and Risks of Translocating Genetic Material among Populations of *Platanthera leucophaea* in Illinois.

Background

A basic principle of conservation genetics is that genetic diversity supports evolutionary potential. The amount of genetic variability present in a population plays an important role in its long-term survival. If a population does not exhibit genetic diversity, the population may not be able to adapt to changing conditions in the future environment. When populations are strongly differentiated, this usually indicates that they are highly isolated and pollinators and /or seeds are not moving between them. Plant populations that are isolated and small are more vulnerable to loss from stochastic events, which can result in a loss of genetic diversity from the species. Inbreeding depression (the reduced fitness in a given population as a result of breeding of related individuals) can result from self-fertilization and from mating between genetically similar individuals. In flowering plants, any self-compatible species is potentially at risk of inbreeding depression (Wallace 2003, p. 235). Inbreeding depression can vary across life history stages (Dudash 1990, Husband and Schemske 1996) and the population impacts of inbreeding depression may vary depending on which life history stage is most negatively affected (Kittleson and Maron 2000). The negative consequences associated with inbreeding result from increased homozygosity within inbred individuals (Wallace 2003). Inbreeding can increase the risk of extinction if increased homozygosity reduces reproductive output in naturally outcrossed populations (Wallace 2003). In general, the higher the genetic variation within a population, the less likely it is to suffer from inbreeding depression which can result not only from self-fertilization but from mating between genetically similar individuals. It is also important to consider that if a population lacks diversity, it may suffer inbreeding depression or it may have effectively purged deleterious alleles and be surviving and reproducing quite effectively (Havens 2011, pers. comm.).

To move germplasm from one population of *P. leucophaea* to another, or from distant or ecologically distinct environments, might, at least temporarily, decrease the fitness of the receptor plants by diluting the genes specific for local adaptations (Cremieux *et al*. 2010). Repeated introductions over several years may counteract local adaptation and continue to negatively impact the fitness of local populations (Keller *et al.* 2000). However, in the absence of further genetic supplementation, the effects of dilution of local adaptation may be likely to decrease over time (Cremieux *et al.* 2010).

Since 1993, we, the U.S. Fish & Wildlife Service, with many dedicated volunteers and partners, have been cross-pollinating the eastern prairie fringed orchid (*Platanthera leucophaea)*, by hand, within Illinois populations (one plant from one site crossed with another plant from the same site). The initial decision to hand-pollinate *P. leucophaea* was driven by the fact that most populations are small (measured by number of flowering plants), and yet appear to rely on seed production for their maintenance (Recovery Plan 1999, p. 20). In addition, it is believed that smaller populations might be limited in pollinator visits and subsequent volume of seed

production (Recovery Plan 1999, p. 28). Most *P. leucophaea* populations in Illinois occur as part of a fragmented landscape and most of these populations would be considered small (< 50 plants). To our knowledge, no translocation of genetic material between populations of *P. leucophaea* has taken place in Illinois.

P. leucophaea is a self-compatible species, meaning pollen from the flower of one plant can pollinate that same flower or another flower on the same plant and result in seed capsule formation with seed. However, self-fertilization in *P. leucophaea* has been shown to result in a lower percentage of viable seeds (Bowles *et al.* 2002). Using the Wadsworth Prairie and Abbott Park *P. leucophaea* populations, both in Illinois, Bowles *et al.* (2002) examined the crossing effects of *P. leucophaea* on the production of viable seed (Bowles *et al.* 2002). This field study compared self-pollination (1 Wadsworth plant and 1 Abbott plant), outcrossing within populations (1 Abbott plant and 3 Wadsworth plants), and reciprocal outcrossing between populations (2 plants) (Bowles *et al.* 2002). These pollinations included > 5 flowers per inflorescence (Bowles *et al.* 2002). The seeds that were collected from the mature capsules of the crosses were pooled within each plant, then the numbers of seeds containing round distinct embryos were counted (Bowles *et al.* 2002) as viable. This study showed that within-population outcrosses of *P. leucophaea* produced, on average, 50% viable seed, but between-population crosses produced, on average, 70% viable seed.

Bowles *et al.* (2002) suggests that in *P. leucophaea*, inbreeding depression may have "cascading effects" by decreasing the percentage of capsules formed, decreasing the percentage of viable seeds within those capsules, and also decreasing the percent germination of those seeds. Bowles *et al.* (2002) further speculates that the amount of inbreeding in small *P. leucophaea* populations could be greater than in large populations because opportunities for outcrossing may be less in small populations.

In an effort to offset potential negative effects of self-crossing and crossing between related and genetically homogenous individuals within isolated populations of *P. leucophaea*, we are considering whether to cross-pollinate *P. leucophaea* between populations (one plant from one site crossed with one plant from another site in Illinois) to increase the genetic diversity of *P. leucophaea* populations in Illinois. There is a potential risk, however, that this practice might lead to outbreeding depression. We do not currently have evidence from Illinois sites to indicate that either inbreeding or outbreeding depression is occurring.

Havens and Bradford (2001) indicated that the risks of inbreeding and outbreeding depression for *P. leucophaea* would be best assessed through a quantitative genetic experimental design that compares the fitness of offspring resulting from various crossing distances, but because it is not possible to easily, or reliably, germinate and grow *P. leucophaea*, this type of experiment is not an option (Havens 2011, pers. comm.). In lieu of this best experimental option, Havens and Bradford (2001) indicated that studies of molecular genetic variation are often used to estimate population genetic structure and infer whether or not populations harbor variation for ecologically important traits.

<u>Relevant Studies: Illinois, Michigan, and Ohio</u>

Cowden (1993) conducted isozyme analyses of *P. leucophaea* sampled from five different *P. leucophaea* populations throughout the Great Lakes region (Illinois, Michigan, Ohio). She found that *P. leucophaea* populations differed genetically more between populations than within populations; however she did not find any alleles in the *P. leucophaea* populations that were unique to a given population (Cowden 2011, pers. comm.). Cowden (1993) did find that Michigan populations of *P. leucophaea* were very similar to each other, as were Ohio populations, but Michigan and Ohio populations compared to Illinois populations were quite different from each other. The reasons why this population differentiation exists is not known. Pleasants and Klier (1995) speculate that as the species migrated eastward, founder events and genetic drift may have affected gene loci resulting in fixation, or near fixation, of one allele in some populations and fixation, or near fixation, of an alternate allele in other populations.

Relevant Studies: Illinois

Havens and Buerkle (1999) examined the genetic structure of six Illinois *P. leucophaea* populations (Hildy Prairie, Lone Grove (reintroduction), Long Grove, Lyons, Wadsworth, and Wrigley) using randomly amplified polymorphic DNA (RAPD) analysis. Their genetic analysis determined that about 40% of the genetic variation was distributed between populations (Havens and Buerkle 1999, p.3). In other words, the populations were quite different from each other genetically. Even so, they would not recommend augmenting an existing population with seeds or pollen from another population because outbreeding depression could be a risk (Havens and Buerkle 1999, p.3). Results from this study also indicated that there was a trend for smaller populations to retain less genetic variation than larger populations, but it was not statistically significant, and despite this trend, some of the smaller populations still retained a large amount of genetic variation. Based on this, they suggest that inbreeding depression might not be as serious a risk as had been thought (Havens and Buerkle 1999, p.3).

Havens and Bradford (2001) analyzed the genetic structure of eight northern Illinois *P. leucophaea* populations ((Hildy Prairie, Lone Grove (reintroduction), Long Grove, Lyons, Wadsworth, Wrigley, Munson, and Grant Creek) using the DNA technique known as intersimple sequence repeats (ISSR). Results from their 2001 study indicated that about 16% of the genetic variation was distributed between populations (Havens and Bradford 2001). This is a fairly average value relative to other plant species. In addition, they found that 84% of the variation was maintained within populations. The average gene diversity for each population was very similar across populations and was not affected by population size (Havens and Bradford 2001). They concluded that, for these reasons, augmenting an existing population with seeds or pollen from another population would not greatly increase a population's genetic diversity. In addition, because outbreeding depression could be a risk, they would not recommend this management technique (Havens and Bradford 2001). In this study, the average gene diversity (0.11 to 0.19) indicated that a significant amount of variation exists even in the smaller populations. This suggests that inbreeding depression might not be as serious a risk as has been theorized (Havens and Bradford 2001). They also found no significant relationship between population size and genetic diversity, which, although unexpected based on general conservation genetics assumptions, has been previously found in some species. Also, there was no consistent relationship between geographic distance and genetic divergence (Havens and Bradford 2001).

Both *P. leucophaea* population genetic analysis studies conducted in Illinois (Havens and Buerkle 1999, pp. 4-5; Havens and Bradford 2001, p. 6) conclude that because their results showed *P. leucophaea* populations as being quite differentiated, it is more likely that outbreeding depression is a risk and that augmenting an existing population with seeds or pollen from another population would not greatly increase a population's genetic diversity, and therefore they would not recommend this management technique. Finally, both studies conclude that the conservative route would be not to augment natural populations with outside germplasm unless there is convincing evidence of inbreeding depression (Havens and Buerkle 1999, p.5; Havens and Bradford 2001, p.6).

Relevant Studies: Illinois and Wisconsin

Pleasants and Klier (1995) examined allozymes to characterize the genetic variation within and among populations of *P. leucophaea* and *P. praeclara*. The purpose of this study was to examine the levels of genetic variation in the species, the geographic pattern of the variation, and the phylogenetic relationship between the 2 taxa. For purposes of this discussion, only the results from *P. leucophaea* are presented. Samples from 7 populations of *P. leucophaea* were examined from Illinois and Wisconsin. In Illinois, samples were taken from populations located at the Abbott Prairie, Long Grove, Hybernia, Wadsworth Prairie, and Nippersink (now Glacial Park) sites. In Wisconsin, the populations sampled were from the sites of Chiwaukee Prairie, and Pleasant Prairie. They found that about 20% of the genetic variation was due to differences among populations and most of the genetic variation (the other 80%) resides within populations, thus little population differentiation has occurred (Pleasants and Klier 1995, p. 2). In addition *P. leucophaea* does not appear to be genetically impoverished (Pleasants and Klier 1995, p. 2), meaning that its diversity is at comparable levels to that found for other studied orchid species. A measure of the average genetic identity between populations of *P. leucophaea* was 0.981 indicating a high similarity, due, in part, to the fact that there is so little variation in this species (Pleasants and Klier 1995). They hypothesized that this little variation could be due to gene flow between populations resulting from extensive wind dispersal of *P. leucophaea's* small seeds. They concluded that although genetic variation was low in *P. leucophaea*, this was not a cause for concern since many orchids have similarly low levels of variation. There were no populations that supported an unusual amount or kind of genetic variation (Pleasants and Klier 1995, p. 3). Despite the fact that this orchid species is threatened, it does not appear to be any more genetically impoverished than non-threatened orchid species (Pleasants and Klier 1995, p. 13). Thus, in the interest of preserving overall genetic variation in the species, none of the populations sampled, or geographic regions sampled, would warrant special attention (Pleasants and Klier 1995). Pleasants and Klier (1995) stressed that analysis of allozymes only provides a small window on the genome, and ecotypic variation may not be assessed by examining allozyme variation (p.3). In general, they conclude that their results suggest that long term persistence of *P. leucophaea* is unlikely to be limited by levels or pattern of genetic variation (Pleasants and Klier 1995, p.3).

Relevant Studies: Michigan and Ohio

Wallace (2002) conducted a genetic variation study using 3 populations of *P. leucophaea* from Michigan and 7 populations of *P. leucophaea* from Ohio. One objective of Wallace's study was to determine the levels of genetic variation among *P. leucophaea* populations at allozyme and random amplified polymorphic DNA (RAPD) loci (Wallace 2002, p. 38). Because *P.*

leucophaea has a fragmented distribution and variability in population size, Wallace expected genetic differentiation to be high among populations of *P. leucophaea* due to low levels of gene flow and substantial genetic drift (Wallace 2002, p. 38). The seven *P. leucophaea* populations in Ohio were sampled using allozyme analysis. These seven *P. leucophaea* populations in Ohio and an additional 3 *P. leucophaea* populations from Michigan were further sampled using RAPD testing. Both marker types (allozyme analysis and RAPD) showed a pattern of strong differentiation among the populations examined (Wallace 2011, pers. comm.). The allozyme analysis revealed high levels of differentiation between the populations and very low levels of diversity within populations (Wallace 2002, p.37). The inference from this result is that the populations are not exchanging genes, but it is unknown how long this pattern has existed (Wallace 2011, pers. comm.). Wallace's (2002) study indicates that out of the seven *P. leucophaea* sampled populations from Ohio, using the allozyme analysis technique, two of the populations showed no evidence of inbreeding while the other five populations were very highly inbred.

Discussion: Comparison of Molecular Marker Techniques and How Results May Be Affected

Although numerous genetic analyses of *P. leucophaea* populations have been conducted throughout the years and throughout its range, there is difficulty in comparing results from these studies and applying these results to determine whether or not to cross-pollinate between populations in Illinois. The difficulty in the comparison and application of results from all of these studies stems, in part, from the wide range of molecular analysis techniques used in the studies, with their individual benefits and shortcomings, and because not all of the studies sampled populations of *P. leucophaea* from Illinois (Table 1).

Currently, many different molecular marker methods are commonly used for documenting genetic information (Semagn *et al*. 2006, p.2540). These methods include: isozyme or allozyme analysis, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeats (ISSRs), sequence characterized regions (SCARs), sequence tag sites (STSs), cleaved amplified polymorphic sequences (CAPS), microsatellites or simple sequence repeats (SSRs), expressed sequence tags (ESTs), single nucleotide polymorphisms (SNPs), and diversity arrays technology (DArT) (Semagn *et al*. 2006 p. 2540). Each method exhibits its own differences, benefits and limitations. According to the kind of study to be undertaken, the researcher can choose among these molecular techniques, each of which has at least some desirable properties. The studies described in this paper have used one of the following methods: isozyme or allozyme analysis, random amplified polymorphic DNA (RAPD), or intersimple sequence repeats (ISSR), therefore, an overview of only these methods is provided.

Isozymes begin in nature by two general mechanisms, i.e. genetic and epigenetic. Epigenetic origins of isozymes are events such as mutation, polyploidization, or chromosomal aberration (Hoelzel 1991). Epigenetically formed enzymes are not considered isozymes by some researchers; they are considered allozymes which describes an isozyme encoded by allelic genes (Zeidler 2000). Alleles at various loci may also be modified to produce isozymes that are distributed in a population according to Mendelian laws of inheritance (Weeden 1983). Regardless of whether the researcher is analyzing isozymes or allozymes, both methods use electrophoresis as the biochemical technique to detect genetic variation (Zeidler 2000).

Isozyme or allozyme analysis provides a very conservative estimate of the extent of genetic variability within a population. It is a simple, efficient, and inexpensive technique for evaluating the taxonomy, genetics, and epidemiology of plants. Using this technique, the genetic information that can be derived includes the amount of genetic variability (i.e., the percent polymorphism) of a species or population, the amount of heterozygosity, the linkage of specific loci, and genetic maps of the chromosomes (Zeidler 2000). As genetic markers, isozymes are useful for studying population structure, tracing epidemics, and analyzing crosses. The major limitation of isozyme analysis is the low number of markers it provides, because the number of biochemical assays available to detect them is small (IPGRI 2003). Because of this, the percentage of genome coverage is inadequate for a thorough study of genetic diversity. Another disadvantage of isozyme analysis is that the markers are based on phenotype (IPGRI 2003). Because of this, they may be influenced by environmental factors, with differences in expression confusing the interpretation of results (IPGRI 2003). Although isozyme analysis is a source of readily obtainable genetic information which is easily reproduced, the technique does not show polymorphisms which are necessary to determine variation within a group of genetically similar individuals. Also, because differential expression of the genes may occur at different developmental stages or in different tissues, the same type of material must be used for all experiments.

Random amplified polymorphic DNA (RAPD) (Williams *et al.* 1990) and intersimple sequence repeats (ISSR) analysis are based on a polymerase chain reaction (PCR) technique which amplifies the number of copies of a specific region of DNA, in order to produce enough DNA to be adequately tested. Using either of these methods has a number of advantages over isozyme or allozyme analysis. They do not require enzyme activity in a sample and they rely on very small quantities of DNA, which can be extracted from fragments of dried leaf, which is good for field collection and the analysis of herbarium specimens. PCR enables researchers to produce millions of copies of a specific DNA sequence in approximately two hours. In addition, because PCR directly assesses the DNA of an organism, a much wider portion of the genome can be assessed than with isozyme or allozyme analysis, which relies on gene expression. Furthermore, individual PCR-based loci often show much greater allelic variation than isozyme loci, allowing a wider range of population genetic questions to be addressed.

The ease and simplicity of the RAPD technique makes it ideal for genetic mapping, plant and animal breeding programs, and DNA fingerprinting. In many instances, only a small number of primers are necessary to identify polymorphism within species (Williams *et al.* 1990). The RAPD analysis can provide a simple and reliable method for measuring genomic variation, determining taxonomic identity, assessing kinship relationships, and analyzing mixed genome samples. Because it is a relatively straight-forward technique to apply, and the number of loci that can be examined is unlimited, RAPD analysis is viewed as having a number of advantages over other techniques (Lynch and Milligan 1994), however, this technique is less popular due to problems such as poor reproducibility, and difficulty in scoring bands, which can lead to inappropriate inferences.

The inter-simple sequence repeat (ISSR) technique is almost identical to the RAPD technique except that ISSR primer sequences are designed from microsatellite regions and the annealing

temperatures used are higher than those used for RAPD markers (Zeidler 2000). Both techniques, RAPD and ISSR, may be applied to previously unstudied taxa because they are PCR techniques that do not require prior DNA sequence information (Semagn *et al*. 2006). Also, both are normally dominant markers and therefore cannot actually determine the alleles at each locus. Dominance is a disadvantage because it precludes direct analysis of heterozygosity at single loci within individual plants. Inter-simple sequence repeat PCR is a fast, inexpensive genotyping technique based on variation in the regions between microsatellites. ISSRs are random DNA markers, again, similar to the more commonly used RAPDs, but are generally more reproducible and produce more markers per primer (Havens and Bradford 2001). This method has a wide range of uses, including the characterization of genetic relatedness among populations, genetic fingerprinting, gene tagging, detection of clonal variation, cultivar identification, phylogenetic analysis, detection of genomic instability, and assessment of hybridization. ISSR requires fewer experimental steps and is therefore easy to carry out with a low cost-benefit ratio and results in a higher reliability and repeatability than RAPD (Nagaraju et al. 2001; Luque et al. 2002). Both ISSRs and RAPDs are normally dominant markers and cannot actually determine the alleles at each locus. Dominance is a disadvantage because it precludes direct analysis of heterozygosity at single loci within individual trees.

Of the 5 studies reviewed in this paper, two used *P. leucophaea* populations strictly from Illinois, with one of these studies using the RAPD technique (Havens and Buerkle 1999) and the other study using the ISSR method (Havens and Bradford 2001) (Table 1). One study (Pleasants and Klier 1995) used allozyme analysis on *P. leucophaea* populations from Illinois and Wisconsin (Table 1). One study (Cowden 1993) used isozyme analysis on *P. leucophaea* populations from Illinois, Michigan, and Ohio (Table 1). And Wallace (2002) used allozyme analysis on 7 Ohio populations, but used RAPD testing on these 7 populations from Ohio and an additional 3 *P. leucophaea* populations from Michigan (Table 1).

Havens and Buerkle (1999) examined the genetic structure of 6 Illinois *P. leucophaea* populations using RAPD analysis. All 6 Illinois populations were quite differentiated from each other. This may suggest genetic isolation of the populations from each other. Their results also perceived smaller populations retaining less genetic variation than larger populations, but this trend was not statistically significant. Havens and Buerkle (1999) believe that measured molecular variation (randomly amplified polymorphic DNA in this study) should not be correlated with variation in traits that are related to fitness (Havens and Buerkle 1999, p.4). They also believe that molecular techniques do not look at traits that are ecologically important, they do not predict survivability in a changing environment, and they are not a study of adaptation (Havens and Buerkle 1999, p.4).

Havens and Bradford (2001) used the intersimple sequence repeat (ISSR) technique in sampling their 8 Illinois *P. leucophaea* populations. This study determined that 16% of the genetic variation of *P. leucophaea* was distributed between populations with 84% of the genetic variation maintained within populations (Havens and Bradford 2001). They found that the average gene diversity for each population was very similar and was not affected by population size. Havens and Bradford (2001) believe that finding significant ISSR variation in the smaller *P. leucophaea* populations suggests, but does not necessarily mean, that inbreeding depression may not be a serious risk. They also believe that the conservative route is not to augment natural

populations with outside germplasm unless there is convincing evidence of inbreeding depression (Havens and Bradford 2001).

Pleasants and Klier (1995) sampled populations in Wisconsin and Illinois and used only allozyme analysis. They found 20% genetic variation was due to differences among populations, with 80% of genetic variation residing within populations (Pleasants and Klier 1995). They also found little differentiation of the populations sampled with no evidence of *P. leucophaea* being genetically impoverished. No populations supported an unusual amount or kind of genetic variation. Despite the low levels of variation found from this study, the authors note that we should not conclude that the species is lacking the genetic variation required to respond to environmental changes (Pleasants and Klier 1995; Holsinger 1993). In our review of the different genetic analysis methods, it is important to note that the use of allozyme analysis provides a more conservative estimate of the extent of genetic variability within a population as compared with the RAPD or ISSR methods. Of these two methods, RAPD and ISSR, the RAPD method has fallen out of favor with the advent of microsatellite use (ISSR method) (Havens 2011, pers. comm.).

Cowden's (1993) isozyme analyses of 5 *P. leucophaea* populations from Illinois, Michigan, and Ohio showed that the populations differed genetically more between populations than within populations and that no unique alleles were found in a given population (Cowden 1993). Finding unique alleles in any given population may be a reason to augment other populations with these unique alleles, but no unique alleles were found. For each state, the Michigan populations were genetically similar to each other as were the Ohio populations, but comparing the Michigan and Ohio populations to the Illinois populations showed they were quite different from each other (Cowden 1993). Again, in the review of the different genetic analysis methods, it is important to note that the use of isozyme analysis can give a more conservative estimate of the extent of genetic variability within a population as compared with the RAPD or ISSR methods.
Wallace (2002) sampled *P. leucophaea* populations in Michigan and Ohio with the seven *P. leucophaea* populations in Ohio using allozyme analysis and all 10 *P. leucophaea* populations from Michigan and Ohio using RAPD testing. The allozyme analysis (only Ohio populations) showed high levels of differentiation and low levels of diversity (Wallace 2002). Both marker types showed strong differentiation among the populations, meaning the populations are probably not exchanging genes. Wallace (2002) tells us that other factors could explain this lack of diversity. The fixation of different alleles in populations could be the result of origination from divergent source populations, differential selective pressures, and/or genetic drift. Considering the results of this study and the overall low levels of allozyme variation observed in other *Platanthera* species, Wallace speculates that perhaps *P. leucophaea* historically lacks allozyme diversity (Wallace 2002). She goes on to explain that if *P. leucophaea* is a derivative species of the western prairie fringed orchid (*Platanthera praeclara*), as suggested by Sheviak and Bowles (1986), it may be similar to other species in having only a subset of variation found in its parent species, *P. praeclara* (Cheng *et al.* 2000; Cronberg 2000). Wallace (2011, pers. comm.) theorizes that, if we are going to outcross at all, perhaps outcrossing populations that are small may be a better option for long-term sustainability of those populations and that if populations are large enough and exhibit genetic variation, then pollinating within them may be effective. Holsinger (2011 pers. comm.) recommends caution in translocating germplasm from

one population to another unless there is evidence that inbreeding depression is a real threat within Illinois populations of *P. leucophaea*.

We do not know how realistic it is to infer results from studies done in other states on populations of *P. leucophaea* in Illinois, as each population has its own unique genetic structure. However, these studies were conducted on the species of our concern, *P. leucophaea*, and results may provide somewhat relevant information as opposed to studies conducted on another species. We also do not, at this time, have evidence that inbreeding depression is occurring at the *P. leucophaea* populations in Illinois.

Experts' Comments:
Dr. Lisa Wallace (Mississippi State University, Associate Professor) suggests conducting experimental studies to directly estimate inbreeding or outbreeding success rather than population genetic studies (Wallace 2012a pers. comm.). Indicators of inbreeding depression would come from data on seedling recruitment in the populations that have been hand-pollinated. If the seedlings are not doing well, this could be an indicator of inbreeding depression, although environmental conditions could also play a role. Checking the seed viability of those fruits that have been hand-pollinated could be another indicator of inbreeding. Dr. Wallace (Wallace 2012a pers. comm.) also suggested testing seed germination rates (of hand-pollinated plants within a population) in the field by setting up seeds housed in netting and attached to stakes, although she recognizes that this may be impractical as it can take up to 7 years for blooming plants to appear. She also suggests conducting genetic studies across generations if we have knowledge of the new recruits from the hand pollinations or genetic studies prior to the start of the hand pollinations (Wallace 2012a pers. comm.).

Dr. Timothy Bell (Chicago State University, Professor) questions whether there is really a reasonable chance of outbreeding depression if we cross-pollinate between sites. Dr. Bell believes the risk of outbreeding depression in plants is highly overrated and that although geneticists often suggest that outbreeding depression is possible when crossing between populations, there is actually very little empirical evidence for outbreeding depression in plants (Bell 2012 pers. comm.). In contrast, inbreeding depression has been substantially demonstrated in plants. Bowles (*et al*. 2002) crossing experiments indicate lower germination and viability for seeds from selfed crosses, with higher germination and viability for seeds from outcrosses between populations compared to crosses within populations. Dr. Bell (2012 pers. comm.) believes that these results indicate that inbreeding can reduce population viability (through reduced recruitment) and that there is no evidence for outbreeding depression in EPFO populations, at least in the F1 (first) generation. Dr. Bell (2012 pers. comm.) also believes that this is also evidence that translocating genetic material between populations may improve population viability since seed germination and viability is higher when outcrossing between populations.

Dr. Lawrence Zettler (Illinois College, Professor) provided results from Bell, Bowles, and Zettler unpublished data:

In 2000, plants from three EPFO populations separated by as much as 158 km (~98 mi.) were included in an experimental pollination program of either selfing, outcrossed within a population,

or outcrossed between a population. In 2002, the experiment was repeated using populations separated by 309 km (~192 mi.). In 2004, the 2002 protocol was repeated again.

- Seed Viability – in both years (2000 and 2002) seed viability was significantly lower for selfed progeny, but there was no significant difference between outcrossed within and outcrossed between progeny.
- Germination was significantly lower for selfed but did not differ between outcrossed within and outcrossed between.
- Protocorm growth was higher but not significantly greater for outcrossed between at the most advanced of the five growth stages.

Dr. Zettler's opinion: EPFO seeds are light weight, and dust like capable of long distance dispersal. Thus, given the right conditions (e.g. strong winds) this species could be capable of colonizing distant areas through seed dispersal alone. How far would these seeds be capable of traveling? A distance of 100 miles should not be ruled out. In the old days, prairies here were connected and ubiquitous. Presumably the same could be said of the fungus (*Ceratorhiza*). Thus, the probability that such a seed would come to rest in a microhabitat containing the right fungus would be much higher than today. It takes ~ 7 years from seed germination to flowering, and incorporating a long term management strategy that takes into account the pollinator distance capability (=immediate genetic transfer, radius of ~ 50 mi.?) coupled with the seed dispersal capability (genetic transfer every 7 years, radius of ~ 100 miles?), would be something we should consider. Dr. Zettler does not believe this orchid, not its genes, were ever meant to be confined to a small geographical area, especially not an orchid, and especially not an orchid that targets sphingids. He believes we should be cross-pollinating orchids between different populations within 100 miles and stresses that we should not be focusing on "if" anymore, but should focus more on "how frequent" this should take place. Considering that *P. leucophaea* is primarily sphinx moth pollinated, and given that some sphinx moths are known to fly great distances (once read up to 50 miles offshore by pink-spotted hawkmoths), why wouldn't (or shouldn't) we be cross-pollinating plants between different populations, at least occasionally, especially involving populations that would be within "striking distances" of powerful flyers like sphingids? Dr. Zettler suspects that outbreeding depression could possibly play a detrimental role, but his opinion is that this wouldn't be much of a problem unless vast distances were covered. He suggests perhaps using populations within 75 km (~47 mi.) of one another. Dr. Zettler respects the opinion of the "purists" in principle, but in practice, especially in this case, he believes we need to step in given the fragmented habitat conditions that currently exist compared to the bygone era.

Notes from a discussion of this topic at the **2012 Illinois EPFO Volunteer Meeting**

Although this paper mentions that, to our knowledge, we have not moved germplasm (pollen) between sites, we do have some sites that were seeded from more than one seed source and EPFO have appeared. Because of this we do not know if the subsequent EPFO plants are from one or two sources, and, if from two, they are most likely already being hand cross-pollinated or naturally cross-pollinated.

If we decide to cross-pollinate between sites, should microhabitats be matched? Discussion: At, for example, our Wrigley site, we have three microhabitats within what we consider one

population and we are currently hand-pollinating between all three microhabitats. So, we may already be cross-pollinating between microhabitats.

If we decide to cross-pollinate between sites, does distance matter? Historically, couldn't we consider all of our northern Illinois EPFO sites as being connected? We once had contiguous prairie habitat throughout the region. So, does distance between sites really matter if we want to cross-pollinate between sites? The Somme Prairie EPFO population received seed from the Wadsworth site and the Wrigley site (near Waukegan), which are somewhat far from each other. The Gensburg Markham Prairie EPFO site in southern Cook County received seed from Chiwaukee Prairie in Wisconsin and Wadsworth Prairie in Lake County (See Site Seeding Excel Attachment).

If the danger of inbreeding depression is a greater risk to EPFO than outbreeding depression, it is almost irresponsible to continue a practice that may be increasing inbreeding depression.

No one is suggesting that we try to homogenize the genetics across the entire species range; however, some wider distribution of genetic material may be needed. Perhaps we should start exchanging pollen between small sites and other geographically close sites such as the North Branch sites (Bunker Hill, Miami Prairie, Somme Prairie, Somme Nature Preserve, and Wayside Prairie). There was discussion on the assumed fact that the North Branch sites were all started from the same (either one or two) seed source(s) to begin with (See Site Seeding attachment).

Suggestion to start cross-pollinating between sites this field season and somehow mark and collect the seed capsules to determine % of viable seed in the pod and then put back at the site. Doing this would give us more data on this topic.

Should we be considering climate change in our decisions on which sites to cross-pollinate? For example, if our area will be getting warmer, should we move pollen from our southern sites to our more northern sites so as to give the northern sites some of the genetic diversity already adapted to a warmer climate.

<u>Options / Alternatives Analyses with Pros & Cons</u>
The purpose of this white paper is to assemble all relevant information and identify and evaluate potential management options. We are seeking feedback on the following options:

A. We could conduct a more current genetic analysis on both large and small populations of *P. leucophaea* in Illinois. Two of the genetic analyses (Havens and Buerkle 1999; Havens and Bradford 2001) conducted on Illinois populations of *P. leucophaea* used leaf samples collected in 1998 and 1999, 13 and 14 years ago. This type of study may provide the information needed to make a reliable estimate on whether or not inbreeding depression is occurring. However, funding for this type of study may not be readily available. And, if this option is chosen, which type of molecular technique would provide the needed information?

B. In lieu of conducting any genetic analysis from *P. leucophaea* populations in Illinois or rangewide, we could determine if there are field indicators of what inbreeding depression may look like and determine a protocol for volunteer data collection. If monitoring

suggests that inbreeding depression is occurring, that would trigger our reconsideration of pollinating between sites.

C. We could move germplasm (Illinois populations only) between what we determine are "related" populations (i.e. all sites along the North Branch of the Chicago River).

D. We could outcross Illinois populations that are small (<50 plants) and pollinate within populations that are large (>50 plants) and that also exhibit genetic variation, however, we may not know which populations exhibit genetic variation without genetic analysis.

E. We could (2012 field season) cross-pollinate between a few sites that are 50-75 miles apart from each other, keep detailed records on the crosses, and also mark the crossed flower to retrieve the correct capsule after maturity. After collection of the capsule, we would determine the percentage of viable seed. We might want to also set up a within population cross and a selfed cross to collect a capsule from each for comparison of the percentage of viable seed. After the percentage of viable seed is determined, the seed should be taken back to its original site to be dispersed.

The options range from taking a conservative approach of conducting more studies before cross-pollinating between populations to taking the approach that the risk of outbreeding depression is low compared to the risk of inbreeding depression, especially given the historical continuity of habitat. We recognize that proceeding with an outcrossing approach without additional studies may lead to outbreeding depression, and, if so, the process cannot be reversed.
Instead of considering populations of Illinois P. leucophaea as independent of populations in other states, we could consider all P. leucophaea populations nationwide and first conduct a phylogeographic study (Wallace 2012, pers. comm.). Phylogeography is the study of the historical processes that may be responsible for the current geographic distributions of individuals. From this type of study, past events can be inferred such as population expansion, population bottlenecks, vicariance (physical barrier to gene flow or dispersal), and migration. Phylogeography can help prioritize areas of high value for conservation. Phylogeographic analyses can define evolutionary significant units, populations that are considered distinct for purposes of conservation such as current geographic separation, genetic differentiation, or locally adapted phenotypic traits caused by differences in selection. Dr. Wallace (2012b pers. comm.) believes a phylogeographic study could provide a very coarse view of population connectivity, however, if such a study is based on sequence data (as they often are), then it would be looking very far back in time if there does turn out to be geographic structure. This means that we might be able to determine if there's cause to not inbreed populations across the entire range of the species, but we probably wouldn't be able to pick up more localized genetic differences. Dr. Wallace believes this type of study is helpful for establishing species-wide management plans across multiple states, but it is not a good way of answering the immediate question for Illinois EPFO sites of whether or not to cross-pollinate between sites.

<u>Questions:</u>
Are there field indicators of inbreeding depression? If so, what are they and how could we monitor for these?

Are there other studies for rare plant species and the conservation implications of translocating genetic material that might be relevant to our question?

If we start moving germplasm between populations, would we consider the separate populations that we cross as one larger population due to the high rates of gene flow between the populations? If so, three small and perhaps low viability populations may now function as one larger population which, when reassessed, could be of higher viability.

Can genetic analysis be conducted on seeds of a plant as opposed to a leaf sample? If so, could we conduct genetic analysis on the seeds resulting from the crosses between populations in lieu of germinating the next generation to get a leaf sample. Answer from Dr. Lisa Wallace: In theory, genetic analysis could be done on the seeds, but you would need enough starting material to extract DNA. Some people germinate seeds and use that as the material for DNA, but that would probably be difficult with most orchid seeds. Doing a bulk DNA extraction of all seeds in a capsule would not give you the individual-level genotypic data that is needed to estimate inbreeding.

Notes from a group discussion of this topic at the
2012 (March 23) Illinois EPFO Researcher and Landowner Meeting

At this meeting, we sought feedback on the following options.

A. We could conduct a more current genetic analysis on both large and small populations of *P. leucophaea* in Illinois. Two of the genetic analyses (Havens and Buerkle 1999; Havens and Bradford 2001) conducted on Illinois populations of *P. leucophaea* used leaf samples collected in 1998 and 1999, 13 and 14 years ago. This type of study may provide the information needed to make a reliable estimate on whether or not inbreeding depression is occurring. However, funding for this type of study may not be readily available. And, if this option is chosen, which type of molecular technique would provide the needed information?

Group Discussion/Brainstorming:

- A current genetic analysis would not give us any more information than we already have.
- We should first determine what question we are trying to answer and then ask a geneticist to suggest what type of test would tell us the answer. The question is: are our sites currently inbred?
- An entire genome study may not tell us anything we need to know for current management.
- We could explore which genetic molecular technique to use this year and perhaps next year conduct a genetic study.
- We should conduct genetic studies on herbarium specimens to know the genetics of true old specimens.

- Even with genetic testing, you cannot say this particular site is inbred, you could only determine if alleles are missing here or present here. Low genetic diversity does not mean we have inbreeding.
- A genetic test could tell us an effective population size, for example, we are currently under the impression that > 50 plants is a large site, this may not be true.
- Even if you do not think inbreeding is a problem, smaller populations should be outcrossed.
- Best study is that which you could study the offspring of a particular cross, but that's difficult to do for this species.
- There have already been genetic studies that showed selfing of EPFO produced the lowest viable seeds followed by crossing within populations which produced much higher viability of seed, and then crossing between populations produced slightly higher seed viability.
- There have already been too many genetic studies, let's just move on.
- A population with 1 to 5 (or up to 10) individuals consistently over a few years is most likely already inbred and from our census sheet we have many sites like this, and these are the ones that should be cross-pollinated with another site.

B. In lieu of conducting any genetic analysis from *P. leucophaea* populations in Illinois or rangewide, we could determine if there are field indicators of what inbreeding depression may look like and determine a protocol for volunteer data collection. If monitoring suggests that inbreeding depression is occurring, that would trigger our reconsideration of pollinating between sites.

- We could cross-pollinate between populations and analyze the plant size the following year. Perhaps plant size characteristics correlate with population size? This is not true, because plants grown in shade may get leggy and taller stretching for light and it doesn't mean it's better than a shorter plant.

C. We could move germplasm (Illinois populations only) between what we determine are "related" populations (i.e. all sites along the North Branch of the Chicago River).

D. We could outcross Illinois populations that are small (<50 plants) and pollinate within populations that are large (>50 plants) and that also exhibit genetic variation, however, we may not know which populations exhibit genetic variation without genetic analysis.

E. We could (2012 field season) cross-pollinating between a few sites that are ~50-75 miles from each other, keep detailed records on the crosses, and also mark the crossed flower to

retrieve the correct capsule after maturity. After collection of the capsule, we would determine the percentage of viable seed. We might want to also set up a within population cross and a selfed cross to collect a capsule from each for comparison of the percentage of viable seed. After the percentage of viable seed is determined, the seed should be taken back to its original site to be dispersed.

Discussion, Summary, and Next Steps:

The group decided that we should cross-pollinate between sites this field season (2012) using the criteria we have agreed upon:

1) Initial focus should be to cross-pollinate between small populations (1-10 plants consistently over the years).

2) We should cross as closely as we can between similar habitats.

3) Encourage cross-pollination between sites within ~ 50 miles.

4) Using our seed distribution table as guidance, sites that are considered "pure" (defined as either a natural population that never had seed brought into the site, or, an introduced population from one seed source) will be left alone (defined as not bringing any pollen into these populations). These "pure" sites could be used as a pollen source.

5) Consider the deer population in determining which sites to cross-pollinate, for example, some sites have deer populations of 200/square mile (Bunker Hill, Wayside, Miami). We would not want to spend time and energy trying to make these populations highly viable because we'd have to cage each blooming orchid to exclude deer herbivory.

6) We should prioritize populations before we do anything i.e. smallest pops first, crossed with populations within ~ 50 miles.

7) We should follow the recovery plan in trying to cross-pollinate populations where, if they produce larger populations, they would contribute to recovery in the appropriate plant community and physiographic regions needed for recovery from Illinois.

8) Dr. Tim Bell and Cathy Pollack will work together in the next month to determine these priority populations.

9) After we determine a cross-pollination protocol, it will be sent out to all of the landowners.

10) If the protocol necessitates the cross-pollination of Nature Preserve sites, Cathy Pollack will apply for the INP permits.

Table 1: Author of each *P. leucophaea* study described in this paper including the year the study was published, the number of populations sampled, the location, by state, of where the samples were obtained, the type of molecular testing conducted, and the results.

Author of Study	**Year Published**	**Number of Populations Sampled**	**State Where Samples Obtained**	**Molecular Test Conducted**	**Results (pop(s)=population(s))**
Cowden	1993	5	Illinois Michigan Ohio	Isozyme Analysis	1) More genetic differentiation between pops than within. 2) No unique alleles to any given pop. 3) MI pops similar to each other. 4) OH pops similar to each other. 5) MI & OH pops quite different from IL pops.
Pleasants and Klier	1995	7	Illinois Wisconsin	Allozyme Analysis	1) 20% genetic variation due to differences among pops. 2) 80% genetic variation resides within pops. 3) Little differentiation has occurred. 4) *P. leucophaea* not genetically impoverished. 5) No pops supported an unusual amount or kind of genetic variation.
Havens and Buerkle	1999	6	Illinois	RAPD	1) All 6 IL pops quite different from each other. 2) Trend for smaller pops to retain less genetic variation than larger pops, but this was not statistically significant.
Havens and Bradford	2001	8	Illinois	ISSR	1) 16% of genetic variation was distributed between pops. 2) 84% of variation was maintained within pops. 3) Average gene diversity for each pop was very similar and was not affected by pop size.
Wallace	2002	3	Michigan	RAPD	Strong differentiation among the pops.
Wallace	2002	7	Ohio	Allozyme Analysis and RAPD	1) Strong differentiation among the pops. 2) High levels of differentiation and low levels of diversity. 3) Two OH pops showed no evidence of inbreeding, but the other 5 were very highly inbred.

Appendix 4 References

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