Estimating the genetic diversity of the threatened orchid *Platanthera leucophaea* to aid management decisions

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Abstract

The federally threatened orchid *Platanthera leucophaea*, Eastern prairie white fringed orchid, is actively being managed as part of its recovery plan. One of the concerns about the orchid is that small and fragmented populations may suffer from low genetic diversity and the associated problems of inbreeding depression, reduced adaptability to environmental conditions, or reduced pollinator attraction. One management option is to augment gene flow (with seeds or pollen) between different populations to increase genetic diversity, though this has the potential problem of causing outbreeding depression. In order to determine the best course of action for orchid management, one would ideally test directly for inbreeding and outbreeding depression. However, difficulty in reliably growing this species makes that approach impossible so we assessed neutral marker diversity as a surrogate. We studied eight *Platanthera leucophaea* populations in northeastern Illinois in order to estimate their genetic diversity and structure. This was done using the PCR based technique ISSR because of its high sensitivity to low levels of genetic diversity. We found that most populations retained a moderate amount of genetic diversity, regardless of size, and that all but two populations were significantly different from one another. Based on these findings, we do not recommend augmenting existing populations.

Introduction

Natural history

Platanthera leucophaea (Eastern prairie white fringed orchid) is a showy, short-lived perennial member of the Orchidaceae. It is federally listed as threatened (Federal Register, 1989). Currently it is known from 57 populations throughout seven states in the Great Lakes region and generally occurs in mesic to wet prairies, sedge meadows and fens (Bowles, 1999). The species is threatened due to habitat loss, competition from exotic species, over-harvest, and reproductive and demographic factors (Bowles, 1999). *P. leucophaea* is pollinated by night-flying hawkmoths (Sphingidae), but natural pollination events are relatively rare and hand-pollinations are used to produce seed for recovery efforts (Bowles, 1983; Keibler, 1998).

Importance of genetic diversity

Most scientists agree that the amount of genetic variability present in a population plays an important role in its long-term survival. Genetic variation can buffer populations against fluctuating environments, pathogen attack and demographic stochasticity (Huenneke, 1991). Loss of variation can lead to genetic problems such as inbreeding depression, reduction in the species' ability to respond to environmental changes and to a reduction in the number of alleles for ecologically significant loci, such as mating types in self-incompatible species. Determining which populations are particularly depauperate, which are relatively diverse, and which are most isolated genetically can guide management decisions. These data can translate into the identification of genetically robust populations to target for habitat protection and seed collection for banking. They can also be used to identify imperiled populations that are candidates for augmentation by the introduction of new individuals (Haig, 1998). Augmenting gene flow through deliberate movement of pollen and seeds can alleviate inbreeding depression, but may lead to outbreeding depression (a decline in fitness as a result of crossing genetically dissimilar sources).

Testing genetic diversity

The potential risks of inbreeding and outbreeding depression are best assessed through a quantitative genetic experimental design that compares the fitness of offspring resulting from various crossing distances. Unfortunately, *P. leucophaea* is poorly suited for this type of study because we are currently unable to reliably germinate and grow this taxon. 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. In doing so, one makes the critical assumption that multi-locus heterozygosity is correlated with additive genetic variance in quantitative traits. To date, there is not much empirical evidence to support this assumption (Storfer, 1996; Reed and Frankham, 2001) and it is important that DNA studies be interpreted with this caveat in mind.

Materials and Methods

A small amount of leaf tissue (roughly 100 mg.) was collected from plants in each of eight populations of *P. leucophaea* in northern Illinois in 1998 and 1999. The DNA was extracted from each sample and analyzed using a PCR (polymerase chain reaction) electrophoretic technique known inter-simple sequence repeat (ISSR). ISSR is similar to the more commonly used RAPDs (random amplified polymorphic DNAs), but are generally more reproducible and produce more markers per primer (Esselman et al., 1999; Nagoaka and Ogihara, 1997; Qian et al., 2001). All the individuals were run twice with five primers (University of British Columbia primer set #9) that demonstrated variable, clear, and reproducible bands. Gel images were captured with a digital camera and analyzed with the Kodak 1D software package. The data were analyzed by AMOVA (analysis of molecular variation) population genetics software (Schneider et al. 1997; Arlequin ver. 1.1) to describe the amount and pattern of genetic variation.

Results

The five primers produced a total of 33 usable bands, 23 of which are polymorphic (69.7%). We scored 104 different haplotypes from the 158 samples.

Average gene diversity over loci

Populations included in the genetic analysis, their census size the year of leaf collection (1998 or 1999), the number of plants analyzed, and average gene diversity over loci, wh indicates the amount of variation that exists within each population.

Population	Pop. size	Number plants analyzed	Average gene diversity over loci
Hildy Prairie	116 (1998 census)	20	0.113
Lone Grove (reintroduced)	13 (1998 census)	7	0.186
Long Grove	52 (1998 census)	10	0.106
Lyons Wood	91 (1998 census)	18	0.171
Wadsworth	170 (1998 census)	20	0.138
Wrigley	26 (1998 census)	17	0.129
Munson	113 (1999 census)	38	0.126
Grant Creek	34 (1999 census)	28	0.188
		158 total	**Ave 0.145**

Average gene diversity values range from 0.113 to 0.188. While these values are relatively moderate, they do indicate that sign ificant genetic diversity exists within each popul There was no significant relationship between population size and genetic diversity (r^2=0.155) or sample size and genetic diversity (r^2=0.004).

Analysis of Molecular Variance (AMOVA)

AMOVA parallels the traditional analysis of variance framework. AMOVA is used to describe genetic structure and variability amo ng populations. The percent of variation partitioned among populations is the PHIst value. This is analogous to Wright's Fixation index or Fst. PHIst values range from 0 (no differentiation between populations) t (complete differentiation).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	7	52.38	0.30	15.57
Within populations	150	247.74	1.65	84.43
Total	157	300.12		PHIst=0.156

The PHIst value of 0.156 indicates that about 16% of the genetic variation in our sample is distributed between populations and 84% of the variation is maintained within populations.

Matrix of pairwise population PHIst values

Matrix of pairwise population PHIst values (values can vary from 0-1 and are an estimate of genetic distance, higher values indicate more divergence between populations) an geographic distance (in km.) between all the populations in the study.

Pairwise Pop. PHIst values (distance [km.])	Wrigley	Hildy	Lone grove	Long grove	Lyons	Wads-worth	Munson
Hildy	.07 (146)						
Lone grove	.31 (50)	.37 (110)					
Long grove	.17 (28)	.36 (120)	.15 (32)				
Lyons	.08 (4)	.15 (151)	.25 (54)	.16 (33)			
Wadsworth	.00 (17)	.14 (156)	.24 (55)	.10 (37)	.10 (8)		
Munson	.11 (230)	.27 (156)	.23 (184)	.01 (208)	.09 (232)	.06 (230)	
Grant Crk.	.16 (124)	.28 (24)	.10 (95)	.07 (100)	.19 (128)	.12 (132)	.15 (180)

Results ranged from 0.00 to 0.37 with all but two being significantly different at the 0.05 level. These fall within the same general range as other outcrossing plants. A similar study of the federally threatened *Cirsium pitcheri* found pairwise population PHIst values between 0.06 -0.20 (Havens, unpublished data) and Wolfe obtained values of 0.50-0.53 for *Penstemon* species (Wolfe et al. 1998). There was not any significant relationship between geographic distance and genetic divergence in the region sampled, which is relatively small in terms of the overall range of the species (r^2=0.021).

Management Conclusions

We want to emphasize that this is only one set of data to consider when developing a management program for *Platanthera leucophaea*. Molecular marker variation may not be correlated with variation in traits that are related to fitness, although most practitioners assume a relationship between marker diversity and heritable genetic variation. It is important to remember that molecular techniques cannot definitively say whether or not inbreeding or outbreeding depression is likely to occur.

The distribution of genetic diversity from this study indicates the existence of significant genetic diversity in and between each population regardless of population size. Because of this data, unless there is convincing evidence of inbreeding depression, we would not recommend augmenting gene flow between populations.

For restoration projects on properties in the region without current populations of the orchid, there is no compelling molecular evidence to limit seed collection to only the closest population(s). We would suggest that matching the habitat of the donor and recipient site as closely as possible is more important than staying within a certain geographic distance for seed collection purposes

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