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 (draft):

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 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.

Questions:

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) EPFO Researchers and Landowners 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 *P. leucophaea* Populations Sampled**	**State Where Samples Obtained**	**Type of 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.

Citations

Bowles M.L., K.A. Jacobs, L.W. Zettler, and T.W. Delaney. 2002. Crossing Effects on Seed Viability and Experimental Germination of the Federal Threatened *Platanthera leucophaea* (Orchidaceae). Rhodora 104(917): 000-000.

Cheng, Y. P., Chien C. T, and Lin T. P. 2000. Population Genetics of Geographically Restricted and Widespread Species of *Myrica* (Myricaceae). Jounal of Herdedity 91: 61-66. *In:* Wallace 2002. Examining the Effects of Fragmentation on Genetic Variation in *Platanthera leucophaea* (Orchidaceae): Inferences from Allozyme and Random Amplified Polymorphic DNA Markers. Plant Species Biology 17:37-49.

Cowden, N.E.F. 1993. A biosystematic study of *Platanthera ciliaris*, *P. blephariglottis* and *P. Xbicolor*, emphasizing the northern portion of their range. Ph.D. thesis, Miami University, Osford, Ohio.

Cowden, N. 2011. (Lynchburg College) pers. comm. E-mail from Nancy Cowden to Cathy Pollack on 12/02/2011.

Crémieux L., A. Bischoff, H. Müller-Schärer, T. Steinger 2010. Gene Flow from Foreign Provenances into Local Plant Populations: Fitness Consequences and Implications for Biodiversity Restoration. Amer. J. Botany 97(1): 94–100.

Cronberg N. 2000. Genetic Diversity of the Epiphytic Bryophyte *Leucodon sciuroides* in Formerly Glaciated Versus Non-glaciated Parts of Europe. Heredity 84: 710-720. In: Wallace 2002. Examining the Effects of Fragmentation on Genetic Variation in *Platanthera leucophaea* (Orchidaceae): Inferences from Allozyme and Random Amplified Polymorphic DNA Markers. Plant Species Biology 17:37-49.

Dudash, M. R. 1990. Relative Fitness of Selfed and Outcrossed Progeny in a Self- Protandrous Species, *Sabatia angularis* L. (Gentianaceae): A Comparison of Three Environments. *Evolution* 44: 1129–1139. *In:* Kittelson, P. M. and J. L. Maron. 2000. Outcrossing Rate and Inbreeding Depression in the Perennial Yellow Bush Lupine, *Lupinus arboreus* (Fabaceae). Amer. J. Botany 87(5): 652–660.

Havens K. and Buerkle A. 1999. A Population Genetic Analysis of *Platanthera leucophaea* in Northeastern Illinois. U. S. Fish and Wildlife Service, Region 3, Fort Snelling, MN. pp. 2-7.

Havens K. and Bradford K. 2001. A Population Genetic Analysis of *Platanthera leucophaea* in Northern Illinois. Final Report to the Illinois Department of Natural Resources. pp. 2-10.

Havens, K. 2011. (Chicago Botanic Garden) pers. comm. E-mail from Kay Havens to Cathy Pollack on 12/02/2011.

Holsinger, K.E. and Wallace, L.E. 2004. Bayesian Approaches for the Analysis of Population Genetic Structure: An Example from *Platanthera leucophaea* (Orchidaceae). Molecular Ecology 13: 887-894.

Holsinger, K.E. 2011. (University of Connecticut) pers. comm. E-mail from Kent Holsinger to Cathy Pollack on 09/23/2011.

Hoelzel, A. R., Dover, G. 1991. Molecular Genetic Ecology. IRL press at Oxford University Press, Oxford.

Husband, B. C. and D. W. Schemske. 1996. Evolution of the Magnitude and Timing of Inbreeding Depression in Plants. *Evolution* 50: 54–70. *In:* Kittelson, P. M. and J. L. Maron. 2000. Outcrossing Rate and Inbreeding Depression in the Perennial Yellow Bush Lupine, *Lupinus arboreus* (Fabaceae). Amer. J. Botany 87(5): 652–660.

International Plant Genetic Resources Group (IPGRI) and Cornell University 2003. Using Molecular Marker Technology in Studies on Plant Genetic Diversity. Protein-based Technologies: Isozymes. Accessed on-line 01/05/2012.

Kittelson, P. M. and J. L. Maron. 2000. Outcrossing Rate and Inbreeding Depression in the Perennial Yellow Bush Lupine, *Lupinus arboreus* (Fabaceae). Amer. J. Botany 87(5): 652–660.

Luque, C., L. Legal, H. Staudter, C. Gers & M. Wink. 2002. ISSR(inter simple sequence repeats) as genetic markers in noctuids (Lepidoptera). Hereditas 136:251–253.

Lynch, M., B.G. Milligan. 1994. Analysis of population genetic structure within RAPD markers. *Molecular Ecology*. 3: 91-99.

Mulcahy, D.L., M. Cresti, H.F. Linskens, C. Intrieri, O. Silverstoni, R. Vignani, M. Pancaldi. 1995. DNA fingerprinting of Italian grape varieties: a test of reliability in RAPDs. *Advanced Horticultural Science*. 9: 185-187.

Nagaraju, J., K. Reddy, G. Nagaraja & B. Sethuraman. 2001. Comparison of multilocus RFLPs and PCR-based marker systems for genetic analysis of the silk-worm, Bombix mori. Heredity 86:588–597.

Pleasants, J.M. (Iowa State University) and Klier, K. (Univ. of Northern Iowa) 1995. Genetic Variation Within and Among Populations of the Eastern and Western Prairie Fringed Orchids, *Platanthera leucophaea* and *P. praeclara*. A report to the Iowa Department of Natural Resources. pp.1-26.

Semagn, K., Å. Bjørnstad, M. N. Ndjiondjop. 2006. An Overview of Molecular Marker Methods for Plants. Afr. J. Biotechnol. Vol. 5 (25) pp. 2540-2568 29 December.

Sheviak, C.J. and M.L. Bowles. 1986. The Prairie Fringe Orchids: A Pollinator-isolated Species Pair. Rhodora 88:267-278.

Wallace L.E. 2002. Examining the Effects of Fragmentation on Genetic Variation in *Platanthera leucophaea* (Orchidaceae): Inferences from Allozyme and Random Amplified Polymorphic DNA Markers. Plant Species Biology 17:37-49.

Wallace L. E. 2003. The Cost of Inbreeding in *Platanthera leucophaea* (Orchidaceae). American Journal of Botany 90(2): 235-242.

Wallace L. E. 2011. (Mississippi State University) pers. comm. E-mail from Lisa Wallace to Cathy Pollack on 09/23/2011.

Weeden, N. F. 1983. Evolution of Plant Isozymes. *In*: S. D. Tanksley, T. J. Orton [eds.], Isozymes in Plant Genetics and Breeding. Elsevier Science Publishes B. V., Amsterdam, 177–208.

Williams, J.G.K, Anne R. Kubelik, Kenneth J. Livak, J. Antoni Rafalski, Scott V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*. 18(22): 6531-6535.

Wilson, M. R. 1983. Plant Reproductive Ecology. John Wiley and Sons, New York. *In:* Bowles M.L., K.A. Jacobs, L.W. Zettler, and T.W. Delaney. 2002. Crossing Effects on Seed Viability and Experimental Germination of the Federal Threatened *Platanthera leucophaea* (Orchidaceae). Rhodora 104(917): 000-000.

U.S. Fish & Wildlife Service. 1999. Eastern Prairie Fringed Orchid (*Platanthera leucophaea*) Recovery Plan. Fort Snelling, Minnesota. 62pp.

Zeidler, M. 2000. Electrophoretic Analysis of Plant Isozymes. *Biologica* 38: 7-16.

Definitions

Allozymes are enzymes from different alleles of the same gene, whereas isozymes are enzymes from different genes that process or catalyse the same reaction. The two words, allozyme and isozyme, are usually used interchangeably.

Geitonogomy – The pollination of a pistil by pollen from another flower of the same plant.

Genetic drift –The random change of the occurrence of a particular gene in a population; genetic drift is thought to be one cause of speciation when a group of organisms is separated from its parent population. Genetic drift may cause gene variants to disappear completely, and thereby reduce genetic variability. Evolutionary change over generations due to random events in small populations.

Heterozygosity – The state of containing a dissimilar pair of genes for any hereditary characteristic, the result of inheritance of different alleles from parents. Individuals that are heterozygous in as many as possible gene locations have the best performance.

Homozygous – Considered a negative attribute because there is a lack of genetic variation within an individual.

Inbreeding Depression – Reduced fitness in a given population as a result of breeding of related individuals, often the result of a population bottleneck. In general, the higher the genetic variation within a breeding population, the less likely it is to suffer from inbreeding depression.

Population Differentiation – How different one population is from another. The fixation index, F_{ST}, is simply a measure of the diversity of randomly chosen alleles within the same sub-

population relative to that found in the entire population. It is often expressed as the proportion of genetic diversity due to allele frequency differences among populations.

This comparison of genetic variability within and between populations is frequently used in the field of population genetics where values range from 0 to 1. A zero value implies that the two populations are interbreeding freely. A value of one would imply the two populations are completely separate.

Population Variation – Variability in the genetic makeup of a population within or among species; the hereditary variation within and among populations. Lack of variation = high population

Randomly amplified polymorphic DNA (RAPD) - A widely used technique for amplifying anonymous stretches of DNA using PCR with arbitrary primers. RAPD markers are dominant markers and cannot actually determine the alleles at each locus. Allozymes can determine the allele at each locus.