GENETIC VARIABILITY WITHIN AND AMONG POPULATIONS OF THE RARE PLANT SPECIES, ARABIS PERSTELLATA E.L. BRAUN (BRASSICACEAE)

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Genetic Variability Within and Among Populations of the Rare Plant Species, *Arabis perstellata* E. L. Braun (Brassicaceae)

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To the College of Graduate Studies:

We are submitting a thesis written by Nacole Jinks entitled "Genetic Variability Within and Among Populations of the Rare Plant Species, *Arabis perstellata* E. L. Braun (Brassicaceae)".

We have examined the final copy of this thesis for form and content. We recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

NACOLE CHRISTINE JINKS. Genetic Variability Within and Among Populations of the Rare Plant Species, *Arabis perstellata* E. L. Braun (Brassicaceae) (under the direction of DR. CAROL BASKAUF).

Rare species are often found to have limited genetic variability. Potential for adaptation and evolutionary change depends on the degree of genetic variation present within a species, so species with low genetic variability may be at a greater risk for extinction. *Arabis perstellata* is a rare plant species which occurs only in Tennessee and Kentucky, and is federally listed as endangered. This species exhibits a disjunct distribution, with populations in Tennessee occurring roughly 250 miles away from the populations in Kentucky. The genetic variability of this species was estimated by using starch gel and cellulose acetate gel electrophoresis to assay 23 isozymes. Four populations in Tennessee and three populations in Kentucky were sampled. Our results showed no variability within populations, but there was a fixed difference between the two states for one isozyme (IDH). Because all within-state populations were identical at all loci assayed, each within-state population pair had a genetic identity value of 1.000. Due to the fixed difference between Tennessee and Kentucky populations for IDH, each between-state population pair had a genetic identity value of 0.957. At the species level, 4% (1/23) of the loci were polymorphic, with a mean number of 1.043 alleles per locus. At the population level, there were no polymorphic loci within any of the populations, and with only one allele per locus, both the observed and expected heterozygosity was 0. Even when compared with other rare species, *A. perstellata* has an extremely low level of genetic variability. Vigorous conservation efforts are recommended if this species is to be recovered.

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CHAPTER I

INTRODUCTION

Conservation of genetic diversity is important for the long-term evolutionary potential of a species (Barret and Kohn, 1991; Huenneke, 1991). This is because environmental factors often change, and species must adapt to these changes in order to persist. When environmental changes occur, potential for adaptation and evolutionary change depends on the degree of genetic variation present within a species (Hamrick and Godt, 1989; Soltis and Soltis, 1990; Soulé, 1987). Genetic diversity provides the raw material for evolution by natural selection (Fisher, 1930), and the greater the diversity, the more options with which natural selection can work. When there is more diversity, it becomes more likely that beneficial alleles will exist in a population, and this genetic variation can increase the chances of a species' persistence over long time periods (Huenneke, 1991). Even on a shorter time-scale, the loss of genetic variation can have harmful effects on development, survival, and growth rates (Allendorf and Leary, 1986) and the ability to resist disease epidemics (Antonovics, 1984; Beardmore, 1983; Burdon, 1987).

The importance of genetic diversity within a given species has been discussed above, but it is less obvious how the diversity of one species can potentially affect other species around it. All species are members of an ecosystem, existing with other species in a delicate balance. The level of genetic diversity within a species might affect the productivity, growth and stability, and inter-specific interactions within communities and

ecosystem-level processes. For example, levels of diversity in plant species can cause the populations of a particular plant species to fluctuate in numbers, and these fluctuations can influence the fluxes of nutrients in an ecosystem (Hughes et al., 2008) and can have effects across trophic levels (Yoshida et al., 2006). Also, according to a study performed by Booth and Grime (2003), multi-species communities with higher genetic diversity in each species maintained higher species diversity over time than did communities with lower diversity. These studies suggest that the level of genetic diversity for one species might have far-reaching effects. Because so little is understood about the dynamics of most ecosystems, the preservation of species that compose any ecosystem is important.

Many species across the planet are disappearing at an alarming rate, and conservationists are looking for ways to rescue them from extinction. Examining the genetic variability of a species can provide clues about fitness and the possible course of evolution for a species (Weir, 1990). For example, significant variability may indicate that a species is well established and may have the ability to adapt to changes, which could decrease the risk of extinction. Little variation may indicate a number of possibilities, such as a fairly recent disaster (population bottleneck) that caused a decline in the number of individuals, or perhaps that only one or a few individuals of the species has very recently colonized the area (as with the founder effect).

Conservationists should examine diversity at both within-population and among-population levels when making management decisions for a rare species. For example, individuals within a population may be different from each other, but the same variety of alleles that occur in this population may occur in another as well. In this case, there would be high within-population diversity, but low among-population diversity. In such a

case, it would not be necessary to protect all populations to preserve the overall variability of a species, since populations are genetically similar to each other. Or, there may be populations composed of very similar individuals, but the alleles these individuals share might be absent from other populations. In this case, there would be low within-population diversity and high among-population diversity. In this type of situation, it might be necessary to protect almost all populations in order to adequately preserve the overall variability of the species, since different populations contain different alleles. Most species have variability at both of these levels. Although it is uncommon, it is also possible for a species to have no variability at either level (e.g. Lesica et. al., 1987; Peakall et. al., 2003; Waller et. al., 1987).

For this study, I examined the population genetics of a rare endangered plant species, *Arabis perstellata*, because nothing was known about the variability of this species. I wanted to find out if this species has limited genetic variability, like many rare species do, and whether its populations are genetically differentiated. Populations of *A. perstellata* are declining, and more must be known about this species in order to make appropriate conservation and management decisions.

Genetic diversity is affected by a variety of possible evolutionary forces. These forces are discussed in the following section.

Evolutionary Forces Which Act on Genetic Variation

Mutation

Mutation is the primary source of all genetic variation. Variation can only occur if there is a change to the genetic material, and the effects of these changes can be observed over time if the changes are heritable. Allele frequencies usually do not change much over the course of a few generations as a result of mutations, but the cumulative effects over time can be quite dramatic (Hartl, 1981).

Gene flow

Gene flow is the transfer of alleles from one population to another. In the case of plants, gene flow between populations can occur as a result of seed or pollen dispersal. If gene flow is occurring, new alleles may be introduced to populations and thus can increase within-population diversity. While gene flow may increase within-population diversity, it may decrease among- population diversity. The rate of gene flow determines the level of genetic divergence that can occur between populations, and populations that experience high levels of gene flow will be genetically more similar to each other than populations with less gene flow. On the other hand, if gene flow is not occurring between populations, populations will be isolated from each other. They may then become fixed for different alleles, and mutation may produce unique alleles in these isolated populations, causing them to become genetically different from each other.

When species occur in subpopulations, instead of one large, randomly breeding population, the frequency of homozygotes in the region may increase. Sometimes it might be difficult to tell where one population ends and another begins, and researchers may mistakenly count adjacent subpopulations as one large population. There will appear to be a reduction in heterozygotes, relative to Hardy-Weinberg expectations, and this is known as the Wahlund effect (Hartl, 1981). If migration begins between such previously isolated subpopulations, then this gene flow will reduce the frequency of homozygosity (and thus heterozygosity levels will increase, a phenomenon known as "isolate breaking").

Natural selection

Natural selection is an important evolutionary force that acts on phenotypes to cause adaptive changes in a species. If a particular genotype results in a favorable phenotype, the beneficial phenotype will be selected for. Individuals possessing heritable traits that give them a reproductive and/or survival advantage will pass these beneficial traits on to offspring. Directional selection occurs when phenotypes at one extreme end of the range are favored; stabilizing selection occurs when individuals with intermediate phenotypes are favored; and disruptive selection occurs when extreme phenotypes at opposite ends of the range are favored.

Random genetic drift

Random genetic drift is a change in allele frequencies, or even the loss of alleles, due to chance events (Kunin and Gaston, 1993). Genetic drift occurs because the alleles present in offspring are only a random sample of parental alleles, and sometimes chance also determines whether or not an individual will survive to reproduce. Because drift is the result of random rather than adaptive forces, it typically is not beneficial to the viability and reproductive success of a species. Genetic drift may cause genetic variants to be lost completely, and so it reduces overall genetic variability within a population. As alleles are lost, more loci become "fixed" for a single allele throughout the entire population, causing more loci to become monomorphic. Furthermore, unequal frequencies of alleles that may remain at polymorphic loci lead to lower values of expected heterozygosity even at the polymorphic loci (Cole, 2003).

Small populations are prone to random genetic drift. Theory predicts that the smaller the population, the more rapid the loss of diversity due to genetic drift (Wright, 1978). Genetic drift can reduce variation dramatically in small populations because the gene pool is small to begin with, and possible genetic combinations are few. Thus, in small populations genetic traits can become lost or very widespread, with little regard to the adaptive value of alleles (Barret and Kohn, 1991).

One phenomenon that can increase the rate of genetic drift is a population bottleneck. This can occur if there is a disastrous event that causes all but a few individuals to die, or otherwise prevents them from reproducing. Because the rate of genetic drift is inversely

proportional to population size, this sudden decrease in individuals can increase the rate of random genetic drift.

Nonrandom mating

If mating is random, mates will be chosen independently of their genotypes or phenotypes. Some types of nonrandom mating will decrease genetic variability by decreasing heterozygosity levels. One type of nonrandom mating is inbreeding, which is the mating of individuals with relatives. Inbreeding causes a decrease in heterozygosity across all loci in a population. Inbreeding may occur when populations are small, because there are few other individuals with which to cross, and the possible genetic combinations are few. Plants in the proximity of others are typically related, and thus individuals tend to cross with relatives (Beardmore, 1983). Inbreeding in small populations can cause inbreeding depression, which is a reduction in fitness relative to outcrossed individuals because of decreased heterozygosity levels or an increase in expression of deleterious alleles (Lynch, 1990). The most extreme form of inbreeding occurs when some plant species have the capability to fertilize themselves. Plants in small populations tend to be monoecious and self-compatible more often than would be expected by chance (Hamilton, 1990; Kunin, 1991; Longton, 1992). Both self-fertilization and crossing with relatives can be beneficial when individuals are scarce because it may sometimes be the only way a species can persist, but it does result in the loss of heterozygosity (Kunin, 1997). Poor fitness resulting from such phenomena may increase the risk of extinction of a species (Newman and Pilson, 1997; Saccheri et al., 1996).

Mating opportunities can also be limited if individuals are sparsely distributed within a population (Karron, 1991). According to Wright's isolation by distance models, mating is dependent on the distance between individuals and their ability to disperse propagules (Allphin et al., 1998). Plants that rely on insect pollination may be at a disadvantage in a sparse population because pollinators are more likely to visit denser populations. Plants that rely on wind pollination may have trouble persisting because there will be fewer opportunities in sparsely distributed populations for gametes from different individuals to come into contact with each other.

Assortative mating is another type of nonrandom mating that occurs when mates are chosen based on phenotypes. This means that mates will be chosen based on some outwardly observable characteristic(s). In contrast to inbreeding (which decreases heterozygosity levels across all loci), heterozygosity levels will only be affected at loci coding for the particular selected trait. In positive assortative mating, individuals choose mates that tend to be phenotypically similar to themselves for a particular trait, which would cause a decrease in heterozygosity at relevant loci. In negative assortative mating, mates that are phenotypically dissimilar for a particular trait tend to be chosen. This would cause an increase in heterozygosity at relevant loci. While it is obvious how people or other animals could select mates in this way, it is not as apparent how plants might do it. These phenomena can occur in plant populations, however. An example of positive assortative mating can be seen in many flowering plants. The length of flowering time is typically short when compared to the length of the growing season, so only plants that flower at the same time will be able to pollinate each other (Kodric-Brown and Brown, 1979). An example of negative assortative mating can be found in the

polymorphism known as heterostyly. Heterostyly occurs in common primrose (*Primula vulgaris* Huds.) populations, in which there are roughly equal proportions of two different flower types known as pins and thrums. Pins have a tall style and short stamens, and thrums have a short style and long stamens. Pollinators that work high in the flowers will pick up thrum pollen and then deposit it on pin stigmas, and pollinators that work low in the flower will pick up pin pollen and deposit it on thrum stigmas (Darwin, 1877; Yeo, 1975).

Evaluating Genetic Variation

Levels of genetic variation can be evaluated in several ways (e.g. Hartl, 1980). Variability statistics include determining the percent of polymorphic loci, the number of alleles per locus, the proportion of individuals that would be expected to be heterozygous under Hardy-Weinberg equilibrium conditions (see below), and the proportion of individuals that actually are heterozygous. F-statistics provide valuable information about the distribution of a species' genetic variability, and genetic identities (or genetic distances) evaluate the genetic similarity of different populations.

Percent of polymorphic loci

The percent of sampled loci that are polymorphic (P) can be scored by either: (a) finding the proportion of all sampled loci that have more than one allele per locus, (b) finding the proportion of all loci where the most common allele frequency is less than

99%, or (*c*) finding the proportion of all loci where the most common allele appears with a frequency of less than 95%. When multiple populations are sampled, *P* can be calculated as either the mean of the values obtained from each population, or, alternatively, all loci may be considered for the species as a whole (Hartl, 1981).

Number of alleles per locus

Like the P value, the number of alleles per locus can be calculated at either the population level (averaging across all populations) or at the species level (considering alleles per locus for the species as a whole).

Observed and expected heterozygosity

The observed heterozygosity (H_o) is the actual proportion of heterozygotes found in a given population. The expected heterozygosity (H_e) is the proportion of individuals expected to be heterozygotes if the population were in Hardy-Weinberg equilibrium.

For a population to be in Hardy-Weinberg equilibrium, both allele and genotype frequencies must be constant from generation to generation. In order for a population to remain in equilibrium, it cannot be affected by disturbances, such as non-random mating, mutations, natural selection, random genetic drift, and gene flow from other populations. Although a perfect equilibrium is unlikely in nature, this value can still be used as a baseline against which to evaluate genetic change.

The Hardy-Weinberg equation is used to predict the genotype frequencies of the next generation at a particular locus given equilibrium conditions. Assuming conditions of equilibrium, observed allele frequencies can be used to calculate expected heterozygosity levels for a population. Observed genotype data for populations are usually tested (using a goodness-of-fit test) to see if their genotype frequencies at polymorphic loci fit Hardy-Weinberg expectations. The observed and expected heterozygosities can also be used to calculate F statistics.

Wright's F statistics

Developed by Sewall Wright in the 1920's, *F* statistics describe the degree of reduction in the heterozygosity of a population when compared to Hardy-Weinberg equilibrium conditions (Hartl, 1981) and can therefore be used to evaluate the effects on populations of various evolutionary forces, such as non-random mating and genetic drift. Only polymorphic loci (at the species level) are used in the calculations. For each polymorphic locus, *F* statistics use the values of average observed heterozygosity for populations (H_I, or H_o), average expected heterozygosity for populations (H_S, or H_e), and heterozygosity expected if the whole species consisted of one combined population (without the effects of divided subpopulations) (H_T), to evaluate the effects of such forces.

F_{IS} and F_{ST} are the F-statistics usually reported. The inbreeding coefficient (F_{IS}), which is found using the formula $(H_s - H_I)/H_S$, measures the reduction in heterozygosity of individuals relative to Hardy-Weinberg expectations. F_{IS} is often referred to as the inbreeding coefficient because it can indicate the degree of nonrandom mating occurring

in a population due to inbreeding. F_{IS} can also reflect the action of other factors that affect heterozygosity levels, such as the presence of cryptic subpopulations or the action of natural selection promoting heterozygotes. F_{IS} can range from -1 to 1, where a value of -1 would occur if the maximum excess of individuals were heterozygotes when compared to Hardy-Weinberg expectations; a value of 0 would indicate complete random mating; and a value of 1 would indicate total inbreeding (there would be no heterozygotes). The fixation index (F_{ST}), which is found by calculating $(H_T - H_S)/H_T$, compares the expected heterozygosity for a species occurring in subpopulations with the expected heterozygosity for the same species occurring in one large population. F_{ST} values are a measure of population differentiation. F_{ST} indicates the proportion of the species' genetic variability that is due to populations being genetically different from each other (due to random genetic drift or differential selection in different environments). F_{ST} ranges from 0 to 1, where a value of 0 would indicate that none of the variability was due to populations being different from each other (all variability found would be due to within population variation instead), and a value of 1 would indicate that 100% of variability would be due to populations being different from each other (all variability would be due to among- rather than within-population variation). Although not often reported, F_{IT}, which can be determined by the formula $(H_T - H_I)/H_T$,is a measure of the combined effects (e.g. genetic drift and nonrandom mating) (Hartl, 1980).

Nei's genetic identity and genetic distance

The most commonly used method to measure genetic similarity or differentiation of populations is Nei's genetic identity and Nei's genetic distance (Nei,1978). Nei's genetic identity is frequently calculated as an indication of how genetically similar two populations are, and Nei's genetic distance can be calculated to determine how different two populations are. Data can be entered into software programs such as BIOSYS (Swofford and Selander, 1989) to obtain these values. These calculations include all loci, both monomorphic and polymorphic. When allele frequencies between two populations are similar, the genetic similarity approaches 1, while the genetic difference approaches 0. If two populations share no alleles at all, the genetic similarity value is 0 and the genetic differentiation value is infinity.

Rare vs. Common Species

Although there are many exceptions, various trends have been observed when comparing rare species with widespread species. Some rare species seem to be adapted to a relatively narrow range of environmental conditions (Kunin and Gaston, 1993) and may use a more narrow range of resources (Pate and Hopper, 1993), or resources occurring in lower abundances or within a more restricted area (Prober and Austin, 1991). Rare plants also tend to invest less energy in reproduction (for example, having smaller seeds or fruits) as compared with widespread species (Mitchley and Grubb, 1986). In addition, some rare species produce propagules that do not disperse as well as the propagules of many widespread species (Kunin and Gaston, 1993).

Many studies have been conducted to assess the genetic variability of rare species. Overall, rare species tend to show low levels of genetic variation when compared to widespread species, as documented in reviews of isozyme studies by Hamrick and Godt (1989; 1991) that included 473 plant taxa. There are several possible reasons for this lower level of genetic variation typically observed in rare species. Populations of rare plants tend to be small and geographically isolated from one another. This isolation prevents gene flow between populations and also facilitates random genetic drift (Barret and Kohn, 1991). In addition, a situation in which there are few mates available for cross-fertilization may promote inbreeding or self-compatibility (Kunin, 1997). Rare plant species do tend to have breeding systems biased away from outcrossing, which can cause lower heterozygosity levels (Kunin and Gaston, 1993; Soltis and Soltis, 1990). In reviews comparing the genetic variability of hundreds of plant species that are primarily selfing, mixed between selfing and outcrossing, and primarily outcrossing, Hamrick and Godt (1989) and Hamrick et. al. (1979) found that outcrossing species showed much higher levels of variation.

There are always exceptions to the commonly observed trends that distinguish rare species from widespread species. For example, there are rare species that have higher levels of genetic variability than some widespread species, and there are some widespread species with low levels of genetic variability. A major problem is that when comparisons are made between unrelated rare and widespread species, general trends may be obscured because there is potential for factors other than rarity to influence the results. Comparing rare species with widespread congeners – especially ecologically similar congeners with similar mating systems - can help to eliminate other factors that

may cloud results. This is because the differences observed are more likely to be related to the condition of rarity, as opposed to some other factor.

Although studies comparing congeners have been relatively uncommon in the past (Kruckeberg and Rabinowitz, 1985), such comparative studies have become somewhat more common in recent years as awareness of these issues has increased. In one of the earliest examples, Karron (1987) compared 2 pairs of endemic species of *Astragalus* with their widespread congeners for genetic variability, and found that the rare species in the study did in fact have lower levels of genetic diversity than their widespread congeners. As more congeneric population genetic studies have been carried out, several reviews have summarized the findings. Gitzendanner and Soltis (2000) reviewed studies including 34 pairs of rare species and their widespread congeners and found small but significant differences in the genetic variability of the rare and the widespread species. Cole (2003) compared congeners from 56 plant genera, and found significant differences in most of the measures of variability between widespread and rare congeners. These studies also found that, when estimated from F_{ST}, the reduction in gene flow among populations of rare species was significant when compared to widespread species.

Thus the generality that rare species have less variability than widespread species is supported by most studies. Nonetheless, some rare species maintain levels of diversity that are equal to or even exceed that of their widespread congeners. For example, the rare fern *Adenophorus periens* Bishop had much higher variation than its two widespread congeners, *A. tamariscinus* Kaulfuss and A. *tripinnatifidus* Gaudich, even though all are sexually reproducing outcrossing perennials (Ranker, 1994). The two most widespread species of 11 North American *Polygonella* showed reduced within-population gene

diversity as compared to their narrowly endemic congeners (Lewis and Crawford, 1995). However, even though congeners were being compared in this study, the mating systems of the congeners were not the same. The authors speculated that high rates of selfing in the widespread species, and perhaps a large scale migration period during a glacial period, may have been the cause of the lower diversity levels. Population level diversity was similar for the rare shrub *Daviesia suaveolens* Crisp and its widespread congener, *D. mimosoides* R. Br., although species level diversity was lower for the rare species (Young and Brown,1996). In a genetic study that utilized microsatellites as genetic markers rather than isozymes, the rare sunflower, *Helianthus verticillatus* Small was more variable than its widespread congener *H. angustifolius* L. (Ellis, 2008).

Arabis perstellata

Arabis perstellata E.L.Braun, a member of the mustard family (Brassicaceae), is federally listed as endangered because it is rare and occurs in a limited geographical range. This species was first described by E. Lucy Braun (Braun, 1940), and thus its common name is "Braun's Rock Cress". This first description came from samples collected between 1936 and 1939 in Franklin County, Kentucky (United States Fish and Wildlife Service [USFWS], 1995). It should be noted that Love and Love (1976) separated the genus *Boechera* from *Arabis*, based on their finding that members of the *Boechera* genus have 7 chromosomes, while members of the *Arabis* genus have 8. The species in this study, historically referred to as *Arabis perstellata*, was in fact determined to have 7 chromosomes and thus would be named *Boechera perstellata* (E.L. Braun) Al-

Shebhaz. Al-Shebhaz (2003) confirmed morphological differences between the fruits and leaves of the two genera, and so the scientific community is beginning to recognize this proposed separation of the two genera. However because the agencies responsible for managing the populations of the species in this study all referred to it as "*Arabis perstellata*", this study will retain the original genus name.

Description

As described by the USFWS (1995), this species is a small herbaceous perennial, endemic to the states of Tennessee and Kentucky. It has rounded stems and alternate leaves (which have a papery feel, pers. obs.), and due to its pubescence, appears grayish in color. Figure 1 shows the foliage of *A. perstellata*.

Figure 1. Photograph of *Arabis perstellata* leaves (photo courtesy of Carol Baskauf).

Life history

Arabis perstallata flowers from late March to early May, producing small flowers with four petals (Figure 2) that range in color from lavender to white (Center for Plant Conservation, 2008). Each flower has six stamens, 2 of them being shorter than the other four. The two-chambered ovary is elongated and after fertilization develops into a silique. Fruits mature from mid-May to early June. During the first year following germination, a taproot becomes established and a rosette is formed. The following year new flowering stems will emerge from the rosette of the previous season. The rosette will persist for about 2 years, and in subsequent years more growth will occur. The lifespan of *A. perstellata* can be up to 5 years (USFWS, 1995).

Figure 2. Photograph of *Arabis perstellata* flowers (photo courtesy of Carol Baskauf).

Habitat

According to the USFWS (2004), *A. perstellata* habitat consists of undisturbed, closed-canopy, mesophytic and sub-xeric forests with large mature trees. Tree species often associated with *A. perstellata* include *Acer saccharum* Marshall, *Quercus muehlenbergii* Engelm, *Celtis occidentalis* L., and *Aesculus glabra* Willd. *Arabis perstellata* often occurs on the down slope side of tree bases, and because it is sun-intolerant, will occur only in full or partial shade. Natural disturbances seem to facilitate germination, as *A. perstellata* often occurs on animal trails. The plant grows well on open forest floors, with little leaf litter or herbaceous covering. Accumulation of leaf litter is believed to impede germination. Two non-native invasive species, *Lonicera maackii* Rupr.(Amur honeysuckle) and *Alliaria petiolata* Bieb.(European garlic mustard), appear to compete directly with *A. perstellata*. Because *A. perstellata* is a poor competitor, these two invasives have already encroached on much of the habitat and continue to pose a threat (USFWS, 2004), though currently it is a much bigger problem in Kentucky than it is in Tennessee (pers. obs.). Native species also pose a threat, most notably *Toxicodendron radicans* (poison ivy), *Parthenocissus quinquefolia* (Virginia creeper), and *Galium aparine* (bedstraw).

An interesting feature of *A. perstellata* habitats are limestone outcrops. *Arabis perstellata* always occurs on slopes composed of calcium carbonate, calcium, or limestone (USFWS, 2004). Every population studied in this project occurred directly on or very near to outcrops of exposed limestone. Most of the plants found grew on or near limestone -- at the base of an outcrop, out of cracks in the rock, or directly on limestone

where even the smallest amounts of soil or leaf litter had accumulated. Sometimes plants were found a short distance away from the outcrops, but these plants were typically smaller in size.

Occurrence

As of 2004 there were 42 documented populations *of Arabis perstellata,* all occurring in either Tennessee or Kentucky (USFWS, 2004). Thirty-seven occur in Kentucky, within a 200 square mile area. There are six populations listed in Tennessee, but one additional population named Cole Knob was discovered after the 2004 designation of critical habitat by the U.S. Fish and Wildlife Service (A. Bishop, Tennessee Department of Environment and Conservation, pers. comm.). The populations in Tennessee occur on separate hills called "knobs", and these knobs may act as geographical barriers that can restrict gene flow between populations.

Arabis perstellata is found in two separate sections of the Interior Low Plateaus Physiographical Province (USFWS 2004). In Kentucky, it occurs in the Blue Grass Section of this province, and is associated with the Kentucky River drainage system. There are three counties of occurrence in Kentucky: Henry County, Wilson County, and Owen County (Fig. 2). In Tennessee it occurs in the Central Basin Section of the Interior Low Plateaus Province, and is associated with the Stones River and Cumberland River systems. Historically, it occurred in Davidson County, Tennessee (USFWS, 1995), but now it occurs in only Wilson, Rutherford, and Williamson counties. Figure 3 shows sampled populations as dots, and illustrates the disjunct distribution of this species.

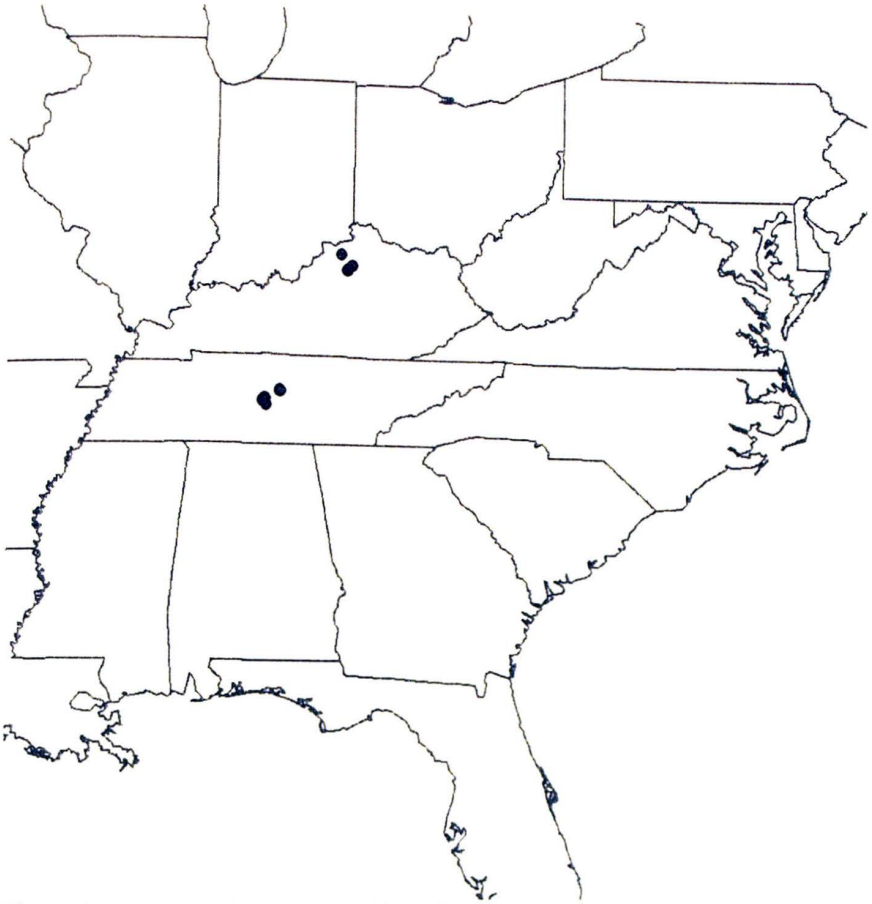

Figure 3. Map showing the sampling sites of *Arabis perstellata.* The dots showing sampling sites also give an indication of the current range of *Arabis perstellata,* which occurs only in the states of Tennessee and Kentucky (see also Figure 4 to view the county distribution for the species) (map courtesy of Steve Baskauf).

Interestingly, both of these sections of occurrence in Tennessee and Kentucky are underlain by strata of Ordovician Age (510 - 438 million years ago) (Quarterman and Powell, 1978). The Ordovician period was characterized by high sea levels, and during this time the states of Tennessee and Kentucky were covered by shallow, tropical seas. The rocks in this area are sedimentary -- chiefly limestone. This is because limestone is composed of calcium carbonate, which comes from the shells and outer casings of marine life. The restricted area and low elevation of solid land prevented erosion, and marine sediments accumulated over time.

The final ruling made by the U.S. Fish and Wildlife Service for the determination of endangered status for this species originally recognized two varieties, based on size and degree of pubescence (Rollins, 1960). "Large rock cress" (*A. perstellata* var. *ampla* Rollins) was found in Tennessee, while "small rock cress" (var. *perstellata*) occurred in Kentucky (USFWS, 1995). However currently, these two varieties are not recognized by the scientific community (Chester et. Al., 2009; USFWS, 2004).

The Tennessee Department of Environment and Conservation's (TDEC) Natural Heritage Division and the Kentucky State Nature Preserves Commission monitor the sizes of the few known populations in Tennessee and Kentucky, but nothing is known about the genetic variability of *A. perstellata*. Before effective conservation efforts can be made to preserve and protect a rare species, the level of genetic variability within the species should first be determined (Beardmore 1983; Frankel 1983; Allendorf and Leary, 1986). Programs to conserve rare plant species need to use appropriate management tools tailored specifically for individual species (Holsinger and Gottlieb, 1988). The degree of variation can provide clues as to how extensive and frequent management intervention should be where it is needed most, and it can be used as a benchmark to measure the changes in fitness of a species over time. In order to obtain a better understanding of how much genetic variability exists within and among *A. perstellata* populations, a population genetics study for this species was conducted.

Methods of Obtaining Data on Genetic Variation

One of the most common methods used in population genetics studies has been protein (especially enzyme) electrophoresis. Different forms of the same enzyme coded for by different gene loci are "isozymes", and different forms of an enzyme coded for by different alleles at a single locus are "allozymes" (although these terms are often used interchangeably). Variations in isozymes are indications of genetic variation in populations (Weeden and Wendel, 1989). Isozyme electrophoresis was one of the early molecular techniques utilized for assaying genetic variability, and it continues to be a useful tool for population level genetic studies. A variety of DNA-based methods for evaluating genetic variability have been devised in more recent years (e.g. amplified fragment length polymorphisms, or AFLPs, microsatellites, etc.), and some of these methods may be able to detect more variability than isozymes. However, DNA-based methods are more expensive than isozymes, and some of the most commonly used DNA-based methods require access to equipment (such as an autosequencer) that is not available at APSU. Furthermore, there is no single best DNA-based method to utilize for population genetic studies; each has its own strengths and weaknesses. For example, AFLPs can result in many genetic markers (fragments from coding or noncoding parts of the genome rather than entire genes), and these are scattered throughout the genome; however, the genetic markers produced are dominant, unlike the codominant isozyme markers. Heterozygosity levels are one of the important statistics desired from most population genetic studies, but no direct count of heterozygotes is possible with dominant genetic markers. Microsatellites, on the other hand, are codominant markers (short tandem repeats, rather than whole genes, are sampled), as well as generally being highly

variable. However, primer development for microsatellites requires extensive preliminary work constructing a genomic library, which does not exist for *Arabis perstellata*.

The genetic variability of *Arabis perstellata* was estimated by using starch gel and cellulose acetate gel electrophoresis to assay isozymes. In sum, this method is relatively fast, effective, and less expensive than DNA-based methods (Schaal et al, 1991), and use of a codominant genetic marker allows a direct count of heterozygotes.

CHAPTER II

METHODS AND MATERIALS

Collection of Plant Material

Collection of plant material began near the end of April 2008, and continued through the summer, with the majority of collections occurring in May. Populations were sampled throughout the range of this species (Figure 4), concentrating on those populations that were larger and /or more geographically distant from each other in order to maximize the chances of detecting genetic variability in the species. Four populations from Tennessee (two from Rutherford County, one from Wilson County, and one from Williamson County), and three populations from Kentucky (two from Franklin County and one from Henry County) were sampled. The Rutherford County populations in Tennessee were Indian Mountain (collected April 24) and Versailles Knob (collected May 18). The other two Tennessee populations were Grandfather Knob in Wilson County (collected on May 7) and Cole Knob in Williamson County (collected on July 10). The Franklin County populations in Kentucky were Camp Pleasant and Rock Cress Hills (both collected on May 30). The Henry County, Kentucky, population was sampled on July 20 by Deborah White and Tara Littlefield of the Kentucky State Nature Preserves Commission, and the leaves were shipped overnight on ice to APSU.

Each population was first surveyed to obtain a rough estimate of the population's size and then sampled in a density-dependent fashion. An attempt was made to sample at least thirty plants from each population. One to three leaves were collected from each plant,

depending on the size of the leaves. Leaves were sealed in labeled Ziploc™ bags and immediately placed on ice. Upon returning from the field, leaves were stored in a refrigerator (~4 to 8° C) in the research lab until use.

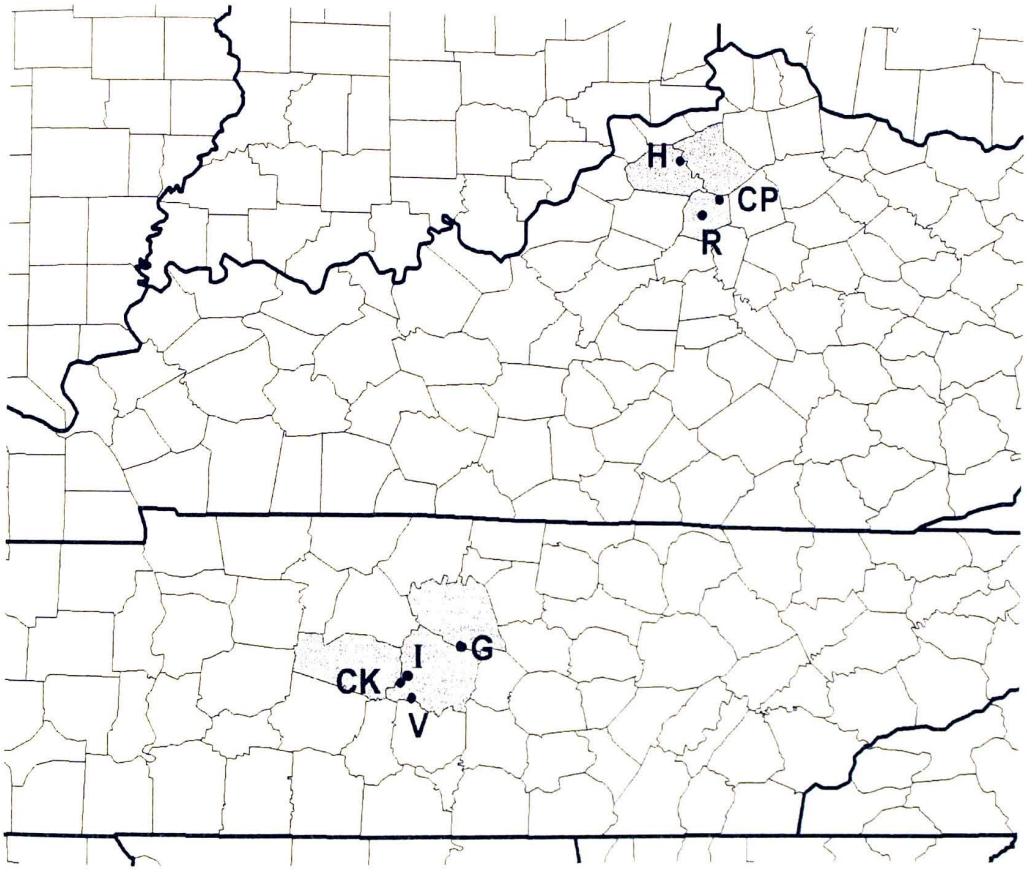

Figure 4. Map showing the range of *Arabis perstellata*, (counties of occurrence are shaded) and the populations sampled for this study. Abbreviations for Kentucky populations are: Henry County (H), Rock Cress Hills (R), and Camp Pleasant (CP). Abbreviations for Tennessee populations are: Indian Mountain (I), Grandfather Knob (G), Versailles Knob (V), and Cole's Knob (CK) (map courtesy of Steve Baskauf).

The sizes of the populations sampled ranged from relatively large (>2,000 individuals) to very small (about 100 individuals) (Table 1). Some populations were denser than others, while others had more scattered individuals occurring over a greater area of land.

All of the populations sampled occurred on private lands, except for one (Rock Cress Hills, KY). Table 1 lists the populations sampled, the number of individuals composing each population, as well as the acreage and ownership of land on which each population occurred.

Table 1: Locations, sizes and ownership of populations of *A. perstellata* sampled for this study.

Population	State	County	# of Individuals[1]	Acreage[2]	Ownership
Indian Mountain	TN	Rutherford	2,000	214	Private
Versailles Knob	TN	Rutherford	200	83	Private
Grandfather Knob	TN	Wilson	200	108	Private
Cole Knob[4]	TN	Williamson	1,300	4.5	Private
Camp Pleasant	KY	Franklin	100	35	Private
Rock Cress[3] Hills	KY	Franklin	200	25	State
Henry County[3]	KY	Henry	100	15	Private

[1]Number of individuals estimated at time of sampling
[2]All acreage information from USFWS (2004), except for Henry County, Rock Cress, and Cole Knob
[3]Henry County and Rock Cress information from Deborah White (pers. comm.)
[4]All Cole Knob information from Andrea Bishop (pers. comm.)

Electrophoresis and Staining for Isozymes

Protein extraction and starch gel preparation procedures generally followed those of Werth (1985) and Wendel and Weeden (1989). Leaves were ground in a cold room by hand using glass test tubes. For each plant, a small piece of leaf material (about the size of a fingernail) was placed in the well of a ceramic spot plate set on a bed of ice, and

ground with Werth's simple extraction buffer (Werth, 1985). For starch gel electrophoresis, wicks cut from filter paper were placed into each well to absorb the leaf homogenate. Twenty-four wicks, each holding the homogenate of one plant, were then loaded onto the cut edge of a starch gel. When placed in an electrical field, molecular components of the homogenate, including enzymes, migrate through the gel towards the node of opposite charge. Components migrate at different rates, and separate out according to their size and charge. Isozymes with similar sizes and charges (and therefore resulting from genes coding for similar amino acid sequences) line up with one another on the gel, while those that differ in size or charge may not. To keep gels cool they were placed beneath a pan of ice, and electrophoresis was carried out in a refrigerator and monitored periodically to ensure that gels did not become too hot. On the far right side of each gel, a bromophenol blue marker indicated when the gel had been run long enough.

Gels were sliced horizontally, and each slice was stained in order to visualize a specific enzyme. Three hundred ml and 400 ml gels were used, from which three and four slices could be obtained, respectively. Each slice contained a complete run of the same samples. Stains have been devised for a large number of enzymes. In general, a suitable substrate is provided, and the product formed by the enzyme can then be visualized directly if it is colored, or indirectly if the product causes a reaction with an added dye.

Electrophoresis protocols, buffer recipes for gels, electrode buffers, and stain recipes generally followed Werth (1985) and Wendel and Weeden (1989). Buffer systems to be used and isozymes to stain for were chosen on the basis of results from earlier isozyme survey work (Baskauf, C.J., pers. comm). Three different starch gel buffer systems were

used: a tris borate EDTA pH 9.1 ("dilute salamander B", Baskauf, 1994), tris borate EDTA pH 8 (Werth, 1985), and morpholine citrate pH 6.1 (Wendel and Weeden, 1989).

Dilute salamander B pH 9.1 was used to assay for acid phosphatase (ACPH), glucose-3-phosphate dehydrogenase (G3PDH), esterase (EST), and phosphoglucomutase (PGM). Tris borate EDTA pH 8 was used to assay for for phosphoglucoisomerase (PGI) and isocitrate dehydrogenase (IDH). Morpholine citrate pH 6.1 was used to assay for adenylate kinase (ADK), fructose biphosphate aldolase (ALD), menadione reductase (MDR), malic enzyme (ME), 6-phopshoglucose dehydrogenase (6PGDH), and malate dehydrogenase (MDH).

Cellulose acetate gels were also used with a tris EDTA borate buffer system (Graham, 1994). Cellulose acetate electrophoresis protocols generally followed Hebert and Beaton (1986). For this method, leaf homogenate is suctioned out of the spot plates and injected into wells. A Super Z™ applicator (Helena Laboratories, Beaumont, Texas) is then used to transfer the leaf homogenate onto a pre-soaked cellulose acetate gel. Once these gels have finished running, they are stained for selected isozymes. This system was used to assay for shikimic dehydrogenase (SKDH), triose-phosphate isomerase (TPI), and glutamate oxaloacetate transaminase (GOT).

Data Analysis

Gels were interpreted according to the position of the isozyme bands on the gel. Each gel was linked by a marker plant to all previous gels. Isozymes with similar amino acid sequences lined up with one another on the gels. If they did not line up, they were

interpreted as having a different genetic makeup for the isozyme in question than those that did line up, and thus to be coded for by a different allele. When lanes did not line up, they were rechecked for consistency, as this implies genetic variation. If an isozyme is monomorphic, bands in all lanes will line up with one another, and this suggests that plants are all homozygous for the same allele for that particular isozyme.

The genetic variability of each population was estimated by calculating heterozygosity levels, the percentage of polymorphic loci, and the mean number of alleles per locus. Wright's (1978) *F* statistics and Nei's (1978) unbiased genetic identity and genetic distance were calculated using the BIOSYS software (Swofford and Selander, 1989). BIOSYS is a multi-purpose program that performs most types of data analysis commonly used by population geneticists (such as computation of allele frequencies, measures of genetic variability, test of fit to Hardy-Weinberg expectations, *F* statistics, heterogeneity chi-square tests, various similarity and distance coefficients, and cluster analysis).

CHAPTER III

RESULTS

In total, 15 enzymes were assayed. Because different forms of some enzymes are coded for by different genes, a total of 24 putative gene loci were assayed. However, only 14 of the enzymes (23 loci) were analyzed in this study because data from esterase could not be consistently resolved for all populations (the enzyme did not stain at all for two populations). Table 2 lists all populations and the number of individuals successfully assayed for each isozyme.

Table 2: Number of *Arabis perstellata* plants successfully assayed from each population for each isozyme. Abbreviations for Tennessee populations are: Indian Mountain (I), Grandfather Knob (G), Versailles Knob (V), and Cole's Knob (CK). Abbreviations for Kentucky populations are: Henry County (H), Rock Cress Hills (R), and Camp Pleasant (CP).

	Populations and sample size per isozyme						
Isozyme	**I (TN)**	**V (TN)**	**G (TN)**	**CK (TN)**	**CP (KY)**	**R (KY)**	**H (KY)**
ACPH	47	33	38	44	32	32	34
ADK	35	34	32	42	32	36	34
ALD	43	29	30	43	31	31	37
EST	24	-----	34	20	32	29	-----
GOT	27	33	31	42	32	33	36
G3PDH	34	33	37	44	31	33	31
IDH	24	33	30	41	36	30	31
PGI	51	33	30	49	30	30	34
PGM	47	30	37	37	29	33	33
MDH	47	33	33	39	31	35	32
MDR	43	25	33	44	34	38	33
ME	47	33	38	39	31	35	39
SKDH	28	33	31	41	32	33	33
TPI	23	33	31	42	32	33	30
6PGDH	47	33	39	44	32	35	44
ACPH	47	33	38	44	32	32	34

One isozyme locus was resolved for each of the following: ACPH, ADK, EST, ALD, G3PDH, IDH, ME, MDR, and SKDH. The following enzymes were coded for by more than one locus (number of loci indicated in parenthesis): GOT (2), PGI (2), PGM (2), MDH (3), TPI (2), and 6PGDH (4).

Of the 23 gene loci assayed, 22 appear to be invariant across all populations of *A. perstellata* in Tennessee and Kentucky. Only one isozyme, isocitrate dehydrogenase (IDH), shows any variability at all for this species. Although this isozyme was invariant among populations within each state, Tennessee and Kentucky populations were fixed for different alleles at this locus.

At the population level, no variability was found. All loci were fixed for only one allele at the population level, and thus, heterozygosity was 0 for every locus. At the species level, 4% (1/23) of the loci assayed were polymorphic, and the mean number of alleles per locus was 1.043 -- all due to the fixed difference for IDH between Tennessee and Kentucky populations.

Because F_{ST} values are calculated using only polymorphic loci, the F_{ST} estimate for *A. perstellata* is based on only one isozyme (IDH) rather than an average of various polymorphic loci. The F_{ST} value estimate for *A. perstellata* is 1.00, indicating that 100% of what little genetic variability the species has is due to populations being genetically different, i.e. populations of the two states are different from each other at this single gene).

Due to the complete lack of polymorphic loci within populations, the inbreeding coefficient, F_{IS}, cannot be estimated.

Genetic identity and genetic distance

Because *A. perstellata* populations within each state are genetically identical at the loci assayed, all population pairs within each state have a genetic identity value of 1.000, (or a genetic distance of 0.000). Although Tennessee and Kentucky populations are very similar genetically, they are not identical. Because of the fixed difference for IDH between populations in the two states, all between-state population pairs have a genetic identity value of 0.957 (or a genetic distance of 0.044). Table 3 shows the genetic identity and genetic distance values for each population pair.

Table 3: Below the diagonal are Nei's (1978) unbiased genetic identity values, and above the diagonal are Nei's (1978) unbiased genetic distance values for each *Arabis perstellata* population pair. Abbreviations for Tennessee populations are: Indian Mountain (I), Grandfather Knob (G), Versailles Knob (V), and Cole's Knob (CK). Abbreviations for Kentucky populations are: Henry County (H), Rock Cress Hills (R), and Camp Pleasant (CP).

Population	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1. TN – I	-----	0.000	0.000	0.000	0.044	0.044	0.044
2. TN – G	1.000	-----	0.000	0.000	0.044	0.044	0.044
3. TN – V	1.000	1.000	-----	0.000	0.044	0.044	0.044
4. TN - CK	1.000	1.000	1.000	------	0.044	0.044	0.044
5. KY - CP	0.957	0.957	0.957	0.957	------	0.000	0.000
6. KY – R	0.957	0.957	0.957	0.957	1.000	------	0.000
7. KY – H	0.957	0.957	0.957	0.957	1.000	1.000	------

CHAPTER IV

DISCUSSION

According to Falk (1991), successful and efficient conservation of genetic variability requires, at a minimum, two conditions: (1) the ability to recognize and define adequate levels of genetic variation where it exists, and (2) the organization of resources to protect and manage variation at these levels.

Genetic Variation

There is a very limited degree of genetic variability within *Arabis perstellata*. Results showed no variability within populations, and variability among populations at only one isozyme – a fixed difference between Tennessee and Kentucky populations. This is of concern because even among many other genetically depauperate species such a dramatically low level of variation is not commonly observed. Table 4 compares the results for *A. perstellata* with the averages calculated for endemic species in Hamrick and Godt's (1989) review of isozyme studies and also with values for several species endemic to Tennessee that have also been assayed for isozyme variability.

Table 4: Genetic variability of *Arabis perstellata* compared with some other rare species, as estimated using isozymes (all values are an average **across all populations**; i.e., an average of within-population variability).

Species	**% Polymorphic Loci**[1]	**Mean # of Alleles Per Locus**	**Heterozygosity (Expected**[1]**)**
Arabis perstellata	0.0	1.00	0.000
Endemic Average[2]	26.3	1.39	0.063
Other Rare Tennessee Species *Echinacea tennesseensis*[3]	23.0	1.3	0.071
Lesquerella stonensis[4]	25.0	1.5	0.076
Lesquerella perforata[4]	41.4	1.6	0.160
Astragalus bibullatus[5]	25.6	1.4	0.064

[1]Any locus having more than one allele is considered polymorphic.
[2]Average of 100 allozyme studies (Hamrick and Godt, 1989).
[3]Baskauf et al., 1994
[4]Baskauf (unpublished data)
[5]Baskauf and Snapp, 1998

Table 5: Genetic variability of *Arabis perstellata* compared with some other rare species, as estimated using isozymes(all values are calculated at the **species** level).

Species	**% of Polymorphic Loci**[1]	**Mean # of Alleles Per Locus**
Arabis perstellata	4.3	1.043
Endemic Average[2]	40.0	1.80
Other Rare Tennessee Species *Echinacea tennesseensis*[3]	27.8	1.5
Lesquerella stonensis[4]	25.0	1.5
Lesquerella perforata[4]	56.3	2.0
Astragalus bibullatus[5]	27	1.4

[1]Any locus having more than one allele is considered polymorphic.
[2]Average of over 81 endemic species (Hamrick and Godt, 1989).
[3]Baskauf et al., 1994
[4]Baskauf (unpublished data)
[5]Baskauf and Snapp, 1998

The Tennessee species in tables 4 and 5 are all rare endemics, and they all occur in areas relatively close to the range of *Arabis perstellata* in Tennessee. *Echinacea tennesseensis* (Beadle) Small (Tennessee coneflower) and *Astragalus bibullatus* Barneby and E.L. Bridges (Pyne's ground plum), are both endemic to the open, shallow-soiled limestone cedar glades of middle Tennessee (Baskauf, 2001). Although the geographic range for these two species is much narrower than for *A. perstellata*, both exhibit greater genetic variation than *A. perstellata*. *Lesquerella stonensis* Rollins (Stones River Bladderpod) and *Lesquerella perforata* Rollins (Spring Creek bladder pod) are both rare Tennessee endemics from the mustard family, yet both species exhibit more variability at isozyme loci than the mustard species in this study, *A. perstellata*.

It is interesting to note that even though all four of these species have fewer known populations, fewer individuals per population, and more limited geographic ranges than *Arabis perstellata* (*L. stonensis* having roughly 20 populations; the other three species having fewer than 10; and all species excluding *L. stonensis* having populations composed of no more than several hundred individuals), *A. perstellata* nonetheless exhibits a dramatically lower level of genetic variability than any of these other rare species (Baskauf and Snapp, 1998; Baskauf, unpublished data; Baskauf et al., 1994). Such low levels of genetic variation may make *A. perstellata* especially vulnerable to extinction. Other factors that make this species vulnerable are its relatively small range, low abundance, and habitat loss due to human development, and competition from exotics (USFWS, 1995).

While such extremely low levels of variability are uncommon, there have been a few cases of similarly genetically depauperate plant species. For example, the rare Torrey

pine (*Pinus torreyana* Parry ex Carriere), which has the smallest population of any pine, had no variability within populations at isozyme loci, although at the species level the two populations were fixed for different alleles at two loci (Ledig and Conkle, 1981). A few species have been found to have no variability for isozyme loci at either the population or the species level: the narrowly endemic species of the St. John River in Maine, *Pedicularis furbishiae* S. Watson, for 22 loci (Waller et al., 1987), the endangered aquatic plant, *Howellia aquatilis* A. Gray, for 18 loci (Lesica et. al., 1987), and a very rare conifer with less than 100 known individuals, *Wollemia nobilis* W.G. Jones, K.D. Hill, & J.M. Allen for 13 allozyme loci. For this latter species, more than 800 AFLP loci were also screened, and no variability was found at any AFLP marker either (Peakall et al., 2003). While these results are interesting, they are unusual. Many isozyme studies have shown that rare species tend to have low levels of genetic variability, but finding no variability at all is uncommon.

The current greatest threat to *A. perstellata* is the loss of habitat, due mostly to the development of new homes and roads. *Arabis perstellata* is also losing habitat to other competitors, both native and non-native species. Native species competing with *A. perstellata* include *Toxicodendron radicans* L. (poison ivy), *Parthenocissus quinquefolia* L.(Virginia creeper), and *Galium aparine* L. (bedstraw). Non-native invasive species that compete with *A. perstellata* include *Lonicera maackii* Rupr. (Amur honeysuckle) and *Alliaria petiolata* M. Bieb.(European garlic mustard). It is important that these invasive species be controlled in *A. perstellata* habitat (USFWS, 1995).

Although the populations occurring in the two states were once considered to be two different varieties (USFWS, 1995), populations from the two states are very similar

genetically. In this study, the only detectable difference was the fixed difference at the IDH locus. With a distance of about 250 miles separating populations of the two states, the Tennessee and Kentucky populations are isolated from each other, and thus random genetic drift is likely responsible for this fixed difference. Nonetheless, population pairs between the two states have high genetic identity values overall (0.957), indicating that populations of plants in the two states are much more similar than they are different.

Distribution of *A. perstellata* in Kentucky is more continuous than the distribution of Tennessee populations (Deborah White, pers. comm.). Tennessee populations occur on separate high hills called "knobs", and this separation could possibly act as a barrier to gene flow. These knobs are separated by areas of now unsuitable habitat such as farm fields, pastures, roads, homes and other forms of human development Because of this discontinuous distribution, one might expect to find alleles which are unique to certain populations, or at least differences in allele frequencies among Tennessee populations. Instead, all population pairs within either state are genetically identical in that they are fixed for the same allele at each locus, resulting in a genetic identity value of 1.000.

Geneticists concerned with long-term viability of rare species typically advocate management that avoids inbreeding and maintains large populations composed of unrelated individuals, in order to maintain genetic variation (Millar and Libby, 1991). In the case of *A. perstellata*, this may prove to be a difficult task, since unrelated individuals within populations could not be identified with the methods used in this study. However, there are other methods with higher resolving power than isozymes that might be able to identify genetically different individuals. For now, maintaining several large populations is necessary.

In a controversial statement, Stebbins (1942) actually suggested the hybridization of rare species with more widespread relatives in order to produce a more robust, adaptable species. Although hybridization may sometimes be the only way to preserve the alleles of an endangered species, genetic assimilation will cause increasing dilution of alleles unique to the rare species. This can occur when a small endemic species is introduced to a more reproductively successful species (Rieseberg, 1991). The more widespread *Arabis shortii* Fernald occurs in a similar habitat to that of *A. perstellata* and is so similar in appearance that the two were once considered as one species. This could make *A. shortii* a potential candidate if this approach were taken with *A. perstellata*, although it is not known if these two species can cross with each other. Regardless of the compatibility of the two species, hybridization is not a good option if any other alternatives are possible due to the dilution and possible loss of unique alleles that would result. Also, it is not known how genetically variable the widespread *A. shortii* is. If *A. shortii* were discovered to have similarly limited variation, this might suggest that rarity may not be the primary factor that is causing the low levels of diversity found in *A. perstellata*.

To prevent *A. perstellata* from declining further in numbers, a vigorous conservation effort is necessary. Habitat protection is necessary for any endangered species (Noss, 1999). Hughes et al. (2008) predict that genetic diversity is most vital in highly variable environments, or those that are subject to habitat loss by anthropogenic change. Because *A. perstellata* is genetically depauperate, critical habitats must be protected to prevent further habitat loss by anthropogenic forces.

Reserves hold promise for preserving ecosystem diversity, but reserves require commitment and funding. Also, once these preserves are established, they will require

attention and expenditure for long-term management (Falk, 1991). The conservation effort for *A. perstellata* is moving in the right direction, because several critical habitat areas have already been designated. These populations are monitored periodically in both states, by the Tennessee Department of Environment and Conservation and by the Kentucky State Nature Preserves Commission. The known populations and designated critical habitats are discussed in the following section.

Critical Habitat Designation

According to the Tennessee Department of Environment and Conservation, there were 56 total populations of *A. perstellata* historically, nine of them in Tennessee and 47 of them in Kentucky (USFWS, 2004). Four of the historical sites in Tennessee and ten in Kentucky no longer have either plants or the physical and biological features required for *A. perstellata* to flourish. According to the USFWS (2004), "primary constituent elements" that sites must possess to be considered as valuable habitat include space for individual and population growth, water, air, light, minerals or other nutritional or physiological requirements, sites for germination and seed dispersal, and habitats that are representative of a species' historical geographical distribution. As of 2004 there were 42 remaining historic locations, 37 of which occur in Kentucky and five in Tennessee (USFWS, 2004). Twenty of these sites had fewer than 50 plants, and therefore are not considered essential for the preservation of *A. perstellata*. In 2004 the Fish and Wildlife Service designated 22 critical habitat sites for *Arabis perstellata*: 14 critical habitat units in Franklin County, Kentucky; three units in Owen County, Kentucky; four units in Rutherford County, Tennessee; and one unit in Wilson County, Tennessee. These

designated sites were all found to have more than 50 plants, and to contain the primary constituent elements for *A. perstellata* (USFWS, 2004). Since 2004, an additional population in Williamson County, Tennessee was discovered, which is called Cole Knob. Because this population was discovered after the critical habitat designation was underway, it is not officially listed as a critical habitat unit. However, 4.5 acres at this site are nonetheless considered critical habitat for this population (Andrea Bishop, pers. comm.). Table 6 lists all habitat units designated as of 2004, as well as the Cole Knob site.

Table 6: Location, ownership, and approximate area of critical habitat units for *Arabis perstellata*. USFWS (2004) is the source of information for all populations except Cole Knob, which has not been officially listed as critical habitat due to its late discovery. Information regarding Cole Knob comes from A. Bishop of TDEC. Populations sampled in this study are marked with an asterisk (the Henry County population is not listed below because it was not designated as critical habitat).

Critical habitat unit	**State**	**County**	**Ownership**	**Acres**
Sky View Drive	KY	Franklin	Private	54
Benson Valley Woods	KY	Franklin	Private	91
Red Bridge Ridge	KY	Franklin	Private	15
Tributary to South Benson Creek	KY	Franklin	Private	25
Davis Branch	KY	Franklin	Private	7
Onans Bend	KY	Franklin	Private	30
Shadrock Ferry Road	KY	Franklin	Private	37
*Hoover Site (Rock Cress Hills)	KY	Franklin	Private	205

Table 6 (cont.)

Critical habitat unit	State	County	Ownership	Acres
Longs Ravine	KY	Franklin	Private	74
Strohlmelers Hills	KY	Franklin	Private	49
U.S. 127	KY	Franklin	Private	27
*Camp Pleasant Branch	KY	Franklin	Private	35
Saufley	KY	Franklin	Private	20
Clements Bluff	KY	Owen	Private	27
Monterey U.S. 127	KY	Owen	Private	30
Craddock Bottom	KY	Owen	Private	57
Backbone North	KY	Franklin	Private	27
Scales Mountain	TN	Rutherford	Private	255
Sophie Hill	TN	Rutherford	Private	132
*Indian Mountain	TN	Rutherford	Private	214
*Grandfather Knob	TN	Wilson	Private	106
*Versailles Knob	TN	Rutherford	Private	83
*Cole Knob	TN	Williamson	Private	106

Once an area is designated as critical habitat, Federal agencies are required to consult with USFWS on any action they carry out, to ensure that their actions do not destroy or

adversely modify the designated area. Critical habitat designation only imposes restraints on Federal agencies, however, so the designation will not protect a plant species if a landowner wants to undertake a project on private land that involves no Federal funding or permits. Because most *Arabis perstellata* populations occur on private lands, it is recommended that the state should make an effort to purchase more property to ensure the protection of these populations. Land owners who do not wish to sell may agree to help preserve the species by leaving the areas undisturbed, and allowing periodic monitoring of the populations.

It is likely that only a very small percentage of the native habitat of *A. perstellata* will be fully protected, so wise management of these areas is crucial. Conservation efforts need to include protection of populations throughout the historic range in both Tennessee and Kentucky. Once protected sites have been established, all preserved areas should be actively managed (e.g., removal of invasive species) and monitored. The boundaries of each area should be clearly marked on the ground and on administrative maps used by conservation agencies (Millar and Libby, 1991). If possible, genetic diversity should be monitored over time, and files should be maintained on the fitness of the populations.

Future Research

As is true for any genetic markers, isozymes represent only a subset of genes that can be observed. Thus, "hidden" variation can go undetected using isozymes alone. It is recommended that a higher resolution method, such as one of the DNA based methods described earlier, be used to determine if any hidden variation does in fact exist.

Because many factors besides rarity can affect genetic diversity (Gitzendanner and Soltis, 2000), it would also be useful to compare the variability of *A. perstellata* with a widespread congener in order to better evaluate the significance of the level of variability detected in *A. perstellata*. The congener, *A. shortii,* was actually once recognized as a variety of *A. perstellata* (Fernald, 1946), but the two are now considered separate species (Kartesz and Kartesz, 1980; Rollins, 1993). The species are very similar because they share a common phylogenetic history, occur in similar habitats, and have a similar size and appearance. *Arabis perstellata* can be distinguished from *A. shortii* by a grayer pubescence which is caused by stellate hairs on its stems and leaves. Therefore, a study comparing the variability of *A. perstellata* to *A. shortii* may be fruitful. An attempt was made to sample some populations of *A. shortii* as part of this study; however, difficulty in acquiring *A. shortii* plants prevented this.

In addition, nothing is known about the mating systems of *A. perstellata*. It would be beneficial to know whether this species is an obligate outcrosser, is self-compatible, or has a mixed mating system. The type of mating system used by a species can have dramatic effects on genetic variability.

Rollins (Rollins, 1960) determined that the chromosome number of *Arabis perstellata* plants in Tennessee was n=7, but the chromosome number for the Kentucky plants is not yet known (USFW,1995). It would be useful to find out if the chromosome number for the plants that occur in Kentucky is the same as the chromosome number for plants in Tennessee.

CHAPTER V

CONCLUSIONS

Genetic variability within *Arabis perstellata* is extremely low. There is no variability among individuals within populations at the 23 isozyme loci assayed, and all populations within a state are genetically identical at these loci as well. Between the two states where this species occurs, there was variability at only one isozyme (IDH). IDH shows a fixed difference between Tennessee and Kentucky populations. *Arabis perstellata* appears to be genetically depauperate, and this is of concern because such a dramatically low level of variation is not commonly observed even among rare species. Low levels of variation can increase a species' risk of extinction because it could result in lower fitness in the short term, and could decrease the chances that the species would be able to adapt to a changing environment in the long term (Frankham, 1995).

Currently, the greatest threat to *A. perstellata* is habitat loss, so habitat preservation is necessary. The U.S. Fish and Wildlife Services has designated several critical habitat units in both Kentucky and Tennessee, and it is important that these units be monitored often and managed to make sure the populations are not declining and that the habitats are not being encroached on by invasive species.

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