THE RESTORATION ECOLOGY OF
FESTUCA ROEMERI

by

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The native prairie and oak savanna of Oregon's Willamette Valley are critically endangered. As part of a restoration effort, I examined the biology of the prairie grass *Festuca roemeri* (Poaceae), which is suspected to have been one of the dominant grass species in this area. I used a common garden study, a pollination experiment and an isozyme survey to assess the mating system and genetic variation. *F. roemeri* can self-fertilize, but can also outcross. Reduced population size may have contributed to the relatively high levels of homozygosity in some populations. Each population was genetically and morphologically distinct and differences in fitness at the common garden site suggest that this variation is adaptive. No evidence of either outbreeding or inbreeding depression was found. Due to the distinctness of the populations, I recommend they not be mixed in future restoration projects, and that more remnant populations be preserved to save the genetic diversity of this species.

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

PREFACE

My thesis is, as the title clearly indicates, grounded firmly in the newly emerged field of restoration ecology. Drawing from all the subdisciplines of biology, including population and community ecology, physiology, genetics, botany, as well as many of the other fields of science, restoration ecology is the study of all aspects of species and communities of species which are relevant to the re-creation or reinvigoration and the long term survival of these communities. It is a relatively new field, with a comparatively small body of literature to its name. Yet, it is arguably one of the most important scientific fields today. This is because we live in a world characterized by change and alteration. In the last few centuries, "humans have emerged as a new force of nature" (Lubchenco 1998), altering and dominating the Earth's surface and biological cycles at a scale and rate unmatched in the history of this planet. I need only mention a few well know statistics, compiled by Luchenco 1998, to make this point clear: "(i) between one-third and one-half of the land surface has been transformed by human action; (ii) the carbon dioxide concentration in the atmosphere has increased by nearly 30% since the beginning of the industrial revolution; (iii) more atmospheric nitrogen is fixed by humanity than by all natural terrestrial sources combined; (iv) more than half of all accessible surface fresh water is put to use by humanity; (v) about one-quarter of the bird species on earth have been driven to extinction; and (vi) approximately two-thirds of major marine fisheries are fully exploited, overexploited, or depleted." Although it may not seem like it, living privileged as we are and doing our best to keep environmental disaster out of sight, our world is fragmented and broken. This is the reality, the demand, to which restoration ecologists are responding. Of all the possible fields to focus on for a senior research project, this is the one to choose.

One question does become immediately obvious, however, when thinking about restoration ecology: Why restore? Of all the possible responses to our current environmental (and social) crisis, why choose to remake the landscape to its "original" state? There are plenty of people confident that human technology and industry can at least substitute for, if not improve on, all the benefits and services that nature has historically provided for humankind. New agricultural techniques and advances in genetic engineering will feed the world, giant filters will clean our air and water, the mysterious forces of chemical and nuclear energy will power our machines, and synthesized medicines and trained medical specialists with all of society's resources at their command will protect us from disease and from death. So why are environmentalists, conservationists, and restoration ecologists so insistent that we restore and conserve the original, or the "native" species and ecosystems? What makes "native" so special? To answer this question, it is necessary to understand what "native" really means.

First of all, "native" is a strangely ambiguous and unscientific term. Referring to a plant or an animal as native to a particular region seems to imply that the species of plant or animal has always been in that area. But scientists know that since the evolution of life on Earth, species have come and gone and ecosystems have changed drastically. In the last few decades, scientists have begun to accept that even on a relatively small temporal scale, most ecosystems, rather than being stable climax communities, are dynamic in terms of species composition and structure. Natural disturbances, competition, and fluctuations in the environment keep many communities in a constant state of change and succession. It is often assumed that a species is native if it existed in a region before the arrival of human beings. But humans have been influencing the distribution of species for thousands of years and who is to say that a species which altered its distribution because of humans (e.g. a weed brought by settlers of a new region in a pasture seed mix) cannot become naturalized and eventually be considered native. Michael Pollan, in his book *Botany of Desire*, questioned whether the apple tree, which was brought to North America by settlers from Europe, should not be considered native: "So what native-plant zealot would dare to challenge the right of such trees to call themselves American now? Their ancestors may have evolved half a world away, but these apples have by now undergone much the same process of acculturation as the people ever did, for the apples reshuffled their very genes in order to reinvent themselves for life in the New World" (Pollan 2001).

Despite the ambiguity, however, the term "native" does imply something significant, both to the labeled species and to humankind. A native plant or animal may not have lived in a given habitat forever, but the time it has spent in that area has allowed it to build a history and a relationship with the local environment and community. It is this history that makes native species so important for restoration. For this history represents possibly thousands of years of evolution, adaptation, mutation, speciation, hybridization, interaction, competition, and co-evolution. This history, besides being a thing of unimaginable beauty and wonder in its own right, is also a resource of inestimable worth to humankind. As an individual species, a given plant or animal may be a potential food source or building material. Chemicals produced by the species to fend off predators or herbivores may offer us the key to a new anti-viral or anti-cancer agent. Within its chromosomes and genetic structure may be a gene to protect our crops from pests or help us resist disease. Each native species is also a part of a native community and ecosystem and so is part of maintaining all the functions that an ecosystem performs and on which we have always depended. Such functions include "the purification of air and water; mitigation of floods and droughts; detoxification and decomposition of wastes; generation and renewal of soil and soil fertility; pollination of crops and natural vegetation; control of the vast majority of potential agricultural pests; dispersal of seeds and translocation of nutrients; maintenance of biodiversity, from which humanity has derived key elements of its agricultural, medicinal, and industrial enterprise; protection from the sun's harmful UV rays; partial stabilization of climate; moderation of temperature extremes and the force of winds and waves; support of diverse human cultures; and provision of aesthetic beauty and intellectual stimulation that lift the human spirit" (Lubchenco 1998). All of these functions are essential for our survival and most, if not all of them we have yet to fully understand, let alone replace or duplicate with our own technologies.

And so here we are, faced with a dilemma of epic proportions, and I am writing my thesis on one grass in a tiny valley in a small inconsequential corner of the Earth. But as inglorious as it may sound, it is species-specific studies such as this that restoration ecology really needs. Of all the problems plaguing restoration efforts, the most basic is the alarming lack of knowledge and understanding concerning these threatened species.

And without this knowledge, all efforts may ultimately fail, despite our best intentions. So now is the time to put off our academic airs and theoretical snobbery and return to some good old-fashioned natural history work (equipped with all the modern technologies of science, of course). Edward Wilson, in a short editorial on the "Future of Conservation Biology," said that "in order to care deeply about something important it is first necessary to know about it" (Wilson 2000). This is exactly what I have tried to do in this thesis, and I hope that it will serve as a model to others who wish to take their undergraduate education and make a difference.

INTRODUCTION

The prairie grass *Festuca roemeri* (Poaceae) is native to the Willamette Valley. Like many native species of grasses and shrubs in the Willamette Valley, *F. roemeri* is critically endangered due to habitat loss and competition with invasives. Historically, grass prairies and open oak savannas dominated the Willamette Valley. These areas were kept open and free of most woody shrubs and tree species because of frequent fires that were set by the native Kalapuyan people and also occasionally by lightning (Clark and Wilson 2001). Much of this habitat has been lost to urbanization and agriculture. What remains is threatened by the invasion of non-native species and the succession of shrubs and trees such as Douglas-fir (*Pseudotsuga menziesii*), which had previously been excluded by fire. It is estimated that less than 1% of the original prairie habitat now remains (Clark and Wilson 2001).

Efforts are currently under way to restore some areas with this habitat and to try to reintroduce historic fire regimes back into the ecosystem. These efforts are hampered, however, by the lack of basic biological and ecological information for many of these native species and their natural ecosystems. *F. roemeri* was a dominant grass in the historic prairies of the Willamette Valley (B. Wilson, personal communication), yet publications on its biology are almost nonexistent. Such basic information as breeding system and self-compatibility are still unknown. Without a more complete understanding of this and other native species, restoration will be difficult and much time and effort may be wasted. For this reason, it was the purpose of my thesis project to study *F. roemeri* and to try to answer some of the basic questions that are pertinent to its restoration. Over the last eight months, I tried to determine the breeding system of *F. roemeri*, to study qualitatively and quantitatively the genetic variation that exists within and between remaining populations of *F. roemeri* in the Southern Willamette Valley, and to test for evidence of outbreeding depression in crosses between individuals from different populations. This information will help inform current and future restoration projects. In particular, these data will help to determine whether the transplanting of *F. roemeri* from other sources to augment populations at Mt. Pisgah will have negative consequences for the long-term survival and growth of the local population. My project will also inform the conservation efforts of the Nature Conservancy at their Willow Creek restoration site.

Plant Breeding Systems, Population Genetics, and Outbreeding Depression

Plant breeding systems is not a simple topic. Unlike vertebrates, which primarily reproduce sexually by cross-fertilization, plants exhibit an astonishing diversity in reproductive systems and strategies. Plants may have a single sex or both sexes represented on a single genet, or individual. They may be primarily outcrossing, or fertilized by the pollen of another genet, or they may be primarily self-pollinating, or inbreeding. Some species of plants are self-incompatible, so that self-fertilization is impossible. Finally, many plants reproduce asexually, either through vegetative reproduction or through agamospermy, where pollen is not required for seed set. Some of these breeding systems are the direct consequence of the sexual structure of the species. However, many other factors, such as population size and pollinator behavior can influence breeding system (Richards 1986).

There are many ways that the sexual organs can be distributed with in plant species. In dioecy, the system most analogous to humans and the majority of vertebrates, each individual of a species has only one type of sexual organ, while in hermaphroditism, each flower on a plant has both the male and the female sex organ. Only about four percent of angiosperms are dioeceous, compared to the 72 percent that are hermaphrodites (Richards 1986). The sexual structure of a plant species has a strong influence on its breeding system. Dioeceous species cannot self-fertilize and so are always outcrossed (although the degree of outcrossing can be variable). Hermaphrodites, on the other hand, can be selfing, outcrossing, or a combination of both, so that knowing that a plant is hermaphroditic actually tells us very little about its breeding system. Often, the degree of selfing in hermaphroditic species depends on the degree of spatial separation between the two sex organs, and also the temporal separation of the maturation of the male and female parts (Richards 1986).

There are several important reasons to study breeding systems in plants. First, as I have already suggested, breeding systems can greatly affect gene flow and, consequently, the genetic structure of a population. In a population of dioeceous plants, for example, where all plants are obligately outcrossing, we would expect to find a high degree of heterozygosity at loci with multiple alleles, assuming that the population is not too small or is not too isolated from other populations (see below). We would also expect that genetic diversity within populations would be as high as or higher than diversity between populations. In other words, for a given group of plants that exist as part of a larger metapopulation, the genetic variability between individuals within that group will be just as high as the genetic variability between individuals of this group and individuals of another group. For a species that is primarily self-fertilizing, such as a hermaphrodite where sex organs mature at the same time in the same flowers and the pistils and stamens are located very close to one another, there will probably be a high degree of homozygosity at each locus, since continual inbreeding (self-pollination or pollination by a close relative) would lead to fixation of alleles. In this case, between-population genetic diversity should be higher than within-population genetic diversity, because between-population gene flow would be limited and in each population, different alleles would become fixed. Of course, in a species that primarily reproduces asexually, each offspring will be identical to its mother and we should expect to see a pattern of fixed heterozygosity. Asexual individuals will have almost no homozygous loci, since mutations leading to different alleles cannot easily be eliminated in an asexual organism (Smith 1989). As with self-fertilization, between-population diversity for an asexually reproducing species will be much higher than within population diversity.

Often times, when studying the breeding system of a species, it is necessary to work backwards, by looking at the genetics of a population and then inferring the breeding system. The first stage is to determine whether the individuals in the populations are primarily homozygous at different loci or whether they are primarily heterozygous. Homozygosity at the majority of loci would indicate selfing or close-relative mating, while heterozygosity in at least some of the loci would indicate either outcrossing or asexual reproduction. As I mentioned above, if the plant is asexually reproducing, there will be few, if any, homozygous loci. In the case that all loci are heterozygous, progeny arrays (offspring) need to be looked at to distinguish between asexual and outcrossing systems. Segregation of alleles would indicate outcrossing, since in an asexual species, the progeny would possess the same genotypes as the mother and paired alleles would remain paired.

A good way to understand population genetic structure is through the concept of Hardy-Weinberg Equilibrium. Hardy-Weinberg Equilibrium (HWE) refers to a population of infinite size, with random distribution of individuals and completely random mating (panmixis). Under these conditions, the genotype frequencies at any given generation for a locus with two alleles are described by the following equation:

$$p^2 + 2pq + q^2 = 1$$

where p and q are the allele frequencies in the population (in the case of polyploidy or more than two alleles, a slightly more complicated but still manageable equation can be used). Although the conditions for HWE seem unrealistic, studies have shown that in populations with greater than one hundred individuals and with a high degree of outcrossing, genotype frequencies do not depart significantly from those in the above equation (Richards 1986). Thus HWE is an important tool for studying population genetics. More specifically, if a population is found to have genotype frequencies that do depart significantly from HWE frequencies, then it is probable that the population is not in panmixis (or that selection has occurred). A higher than expected frequency of heterozygotes can indicate that the population is reproducing asexually, either vegetatively or by agamospermy (assuming that there is no strong heterozygote advantage). If heterozygote frequency is lower than expected, then there is probably a high degree of selfing or near-relative mating (inbreeding). Of course, population genetic structure can be influenced by many factors besides breeding system (e.g. pollinator behavior or natural selection) and so genotype frequencies are not conclusive evidence of breeding system. However, when the data from isozyme electrophoresis (see below) are analyzed with HWE statistics, a great deal can be learned.

It is becoming increasingly clear that the study of plant breeding systems and population genetic structure is important for the field of restoration and restoration ecology. Scientists have always acknowledged that variation, both genetic and phenotypic, can exist within a single population of organisms. However, it is only recently that such ideas as the "home-site advantage" hypothesis and outbreeding depression, both of which emphasize the extreme degree of local adaptation and genetic divergence that can often occur in a subpopulation, have begun to receive notable attention in the field of restoration ecology (Montalvo et al 1997, Montalvo and Ellstrand 2000, Hufford and Mazer 2003). Consequently, there is still very little literature on these subjects.

The "home-site advantage" hypothesis is simply that the relative success or fitness of an introduced individual or a population will decrease as the environmental distance to the local native population increases. In other words, the greater the difference between an individual's "home" site and the site into which it has been introduced, the more difficult it will be for that individual to survive and reproduce. Implicit in this hypothesis is the idea that environments are heterogeneous and that subpopulations of a species living in various microhabitats within a heterogeneous landscape will be subjected to different types of selective pressures. These different pressures will lead to varying degrees of subpopulation-specific adaptation and increased genetic variation in the species as a whole. Because the degree and spatial scale of this heterogeneity can vary greatly from landscape to landscape, the degree to which subpopulations of a species differentiate will also vary. In his review article, Waser (1993) found that significant differentiation could occur within as short a distance as a few meters. Although it is less likely, differentiation within a population that is highly outcrossed can still occur.

When a population is highly inbred, self-fertilizing, or geographically isolated, it is very likely that it will become genetically distinct from the rest of the species, even if its environment is similar to that of the rest of the population. In this case, when it is isolation and not habitat heterogeneity that is influencing the population, genetic drift, which is random changes in gene frequency, will be the primary mechanism of genetic differentiation, rather than natural selection. Natural selection may, however, increase genetic differentiation by selecting for co-adapted gene complexes (see below) (Hufford and Mazer 2003). Genetic drift has not been shown convincingly to be an important influence in continuous populations (Waser 1993). The net effect of these two forces, genetic drift and natural selection, is that even within a species, which is often thought of as the indivisible biological unit, there can exist a high degree of variation.

The greater the degree of genetic variation between subpopulations of a species, the greater the potential for outbreeding depression when two of the subpopulations are interbred. Outbreeding depression is the reduced fitness observed in offspring when parents of two genetically distinct populations mate. Inbreeding depression, reduced fitness as a consequence of self-fertilization or close-relative mating, has been well studied since Darwin (Waser 1993), but it is only recently that scientists have begun to look closely at the effects of outbreeding depression. Outbreeding depression is closely linked to local adaptation. Just as there are two main mechanisms that lead to local adaptation, there are also two mechanisms that have been shown to lead to outbreeding depression.

The first mechanism, known as "ecologically" based outbreeding depression, results from the mating of two individuals which are each adapted to different environments (Montalvo and Ellstrand 2001, Waser 1993). The genetic adaptations of each parent will be "diluted" by the genes of the other parent and the offspring of such a mating will not be well adapted to either of the parental environments. The "ecological" mechanism of outbreeding depression is relatively simple to study because its effects can be observed directly in the F1 (first offspring) generation. The second mechanism, "genetically" based outbreeding depression, occurs when the genetic architectures of two populations have significantly diverged as a result of genetic drift (Montalvo and Ellstrand 2001, Waser 1993). Geographic or genetic isolation leads to the fixation of certain alleles in a population. Later, selection will favor alleles at other loci that work well with the fixed alleles. These sets of co-adapted genes are aptly known as co-adapted gene complexes. Mating between individuals with distinct sets of co-adapted gene complexes will lead to the disruption of these favorable intragenomic interactions and possibly to reduced fitness in the offspring. However, since the disruption of the gene complexes may not occur until the F2 generation (after recombination), this mechanism is usually much more difficult and time consuming to study. In fact, the F1 generation may show increased fitness due to heterosis ("hybrid vigor") before outbreeding depression becomes apparent in the F2 generation. Structural differences between the two parental sets of chromosomes can also cause "genetically" based outbreeding depression, although this is more common in interspecific hybrids (Montalvo and Ellstrand 2001).

When studying the effects and mechanisms of outbreeding depression between two populations, it is important to measure the distance between those two populations. There are several ways to quantify this distance, all of which are closely related to the different mechanisms of outbreeding depression. The three common measures of distance are geographic, environmental, and genetic distance (Montalvo and Ellstrand 2001). Geographic distance is simply the measure of physical space separating the two populations in the landscape (e.g. kilometers). Although geographic distance is the easiest of the three to measure, it has been shown to be the least useful in predicting between-population variation, especially in extremely heterogeneous landscapes (Montalvo and Ellstrand 2001). Depending on the degree of habitat heterogeneity and genetic isolation, a given distance of geographic separation can result in large or small amounts of variation between populations. Environmental distance is a measure of the differences between the environments to which the two populations are adapted (Monatalvo and Ellstrand 2001). For example, if population A is adapted to a dry site at a high elevation and population B is adapted to a low, wet, seasonal flooded site, then the environmental distance between the two populations is relatively high. On the other hand, if two populations are both adapted to high, dry elevation sites, the environmental distance is probably low. Two populations can have a high geographic distance and still have a low environmental distance (and vice versa). Finally, genetic distance is a direct measure of the genetic differences between the populations (Monatalvo and Ellstrand 2001). This includes the type and relative frequency of alleles at each locus, the number of loci coding for each gene, the number and size of the chromosomes, as well as other factors. Genetic distance is the most accurate way of comparing variation between populations, but it is also the most time consuming and technically difficult. Population biologists use such techniques as electrophoresis (which I used) or microsatellites to look at genetic distance. One drawback to these techniques is that since they cannot specifically target adaptive variation or important areas of genetic differentiation, the data can be misleading (Knapp and Rice 1998). Genetic distance is especially important in revealing genetic differentiation in the case when two populations are adapted to identical environments but are genetically isolated. If outbreeding depression is observed in the cross between two populations, measuring both environmental and genetic distance between the two populations can help to determine the underlying cause of the reduced fitness.

There is a good deal of empirical evidence showing that outbreeding depression does occur. However, the relative importance of inbreeding and outbreeding depression and the spatial scale on which these two occur can differ significantly between taxa. Most studies indicate that extreme inbreeding (i.e. selfing) and extreme outbreeding (i.e. mating between different species or highly isolated populations of one species) lead to reduced fitness (Waser 1993). At intermediate levels, results are less clear. Many studies show that both inbreeding and outbreeding depression occur and that there is an optimal outcrossing distance for fitness (Waser 1993, Hufford and Mazer 2003, Price and Waser

1979), but this is certainly not always the case. In addition, some studies show that heterosis, or hybrid vigor, can occur in the F1 generation but that oubreeding depression then is evident in the F2 and later generations, while other studies indicate that only outbreeding depression occurs. Reduced fitness of progeny caused by outbreeding depression has been shown to be severe (Waser 1993, Hufford and Mazer 2003) but it is still unclear how long (i.e. how many generations) this reduced fitness will affect a particular population (Hufford and Mazer 2003). More studies are needed to determine which patterns of outbreeding depression are important to restoration (Hufford and Mazer 2003).

Isozyme Electrophoresis

To study genetic variation within and between populations and to help determine breeding system of the plant, I used isozyme electrophoresis. Electrophoresis is a standard process for many different types of scientific studies (Kephart 2003). The process relies on the basic principle that molecules of varying size and chemical composition will migrate at different rates through a matrix, such as a starch gel, when electric current is run through that matrix (Kephart 2003). If a solution of ground plant tissue is loaded into one side of the gel and current is applied, the different molecules within that tissue will migrate across the gel and resolve themselves into a series of bands, with each band representing a particular kind of molecule. In the case of enzymes, the bands can be "stained" by incubating the gel with substrate and cofactors appropriate for the enzyme of interest. Simple chemical dyes can then register enzyme activity and a colored band will appear on the gel representing the migration point of the enzyme (Crawford 1990).

In my study I used electrophoresis specifically to study isozymes. Isozymes are enzymes in an organism that possess the same catalytic properties or functions but which differ slightly in chemical composition or structure (Kephart 2003). When looking at genetic structure, isozymes can be thought of as different alleles coding for slightly different forms of the same enzyme. Different isozymes of the same enzyme usually migrate at different rates through an electric current. Thus, by using electrophoresis and staining for particular enzymes, it is possible to look quite accurately at the genetic diversity in an individual or population of individuals.

In the simplest case, where an enzyme is coded for by only one gene (intragenic isozyme), deciphering the bands produced by electrophoresis and protein staining can be relatively straightforward. If the enzyme is a monomer, meaning it is composed of only one subunit, then the individual can have a total of two possible isozymes of that enzyme, one produced by the paternal set of chromosomes and one produced by the maternal set of chromosomes. Heterozygotes, in this case, will be indicated by two bands. If the enzyme is a dimer, or composed of two subunits, each of which is coded for by the same gene, then the individual can have a total of three different isozymes. One of these will be composed of only maternally inherited subunits, one will be composed of only paternally inherited subunits, and the last will have one subunit from each of the paternal and maternal set of chromosomes. Usually these subunits associate at random within the cells, so that these three combinations will show up as three distinct bands if the paternal and maternal alleles are distinct. Similary, enzymes with four intragenic subunits (tetramers), will show five distinct bands for heterozygotes. Interpreting banding patterns becomes much more complex if, for example, the enzyme is coded for by multiple genes, different loci for the same enzyme are in different cell compartments (e.g. the mitochondria and the cell nucleus), some of the isozymes are not expressed in the plant, or, as is the case with my study organism, the species is polyploid.

By definition, a polyploid organism has three or more sets of chromosomes (Crawford 1990). In contrast to most animals, including humans, are diploid, or have only two sets of chromosomes, one set contributed by each parent. Many plants are also diploid but many are polyploid. Polyploidy usually arises when a normal diploid cell fails to divide into two haploid cells during meiosis. The resulting gamete will have twice the usually number of chromosomes and when sexual reproduction occurs, the offspring, if viable, will have more than the usual two sets of chromosomes. This process can be an important mechanism for speciation because the first generation polyploid offspring is immediately sexually isolated from the parents and the rest of the diploid population.

Polyploids are classified as either allopolyploids or autopolyploids, according to their origin and the nature of chromosomal pairing during reproduction (Soltis and Rieseberg 1986). Autopolyploids usually arise from the mating of two closely related individuals (i.e. intraspecific). The two fused genomes are usually identical chromosomally and similar if not identical genetically (Crawford 1990). Because of this, during meiosis the homologous chromosomes assort randomly and offspring arrays exhibit tetrasomic ratios. For example, if an autotetrapolyploid (an autopolyploid with four sets of chromosomes) with (AAaa) alleles at a particular locus were to self, the resulting offspring genetic ratios would be 1AAAA: 8AAAa: 18AAaa: 8Aaaa: 1aaaa (Soltis and Rieseberg 1986). An allopolyploid, on the other hand, usually results from the mating of two distantly related individuals or the hybridization of two species. In this case, the chromosomes of the two genomes are too different to assort randomly and only homologous chromosomes of the same parental origin may pair up during meiosis (Crawford 1990). Allopolyploids show a pattern of "fixed heterozygosity" because for each locus, alleles from the first parent will always be present along with the alleles from the second parent. For example, a selfing allotetrapolyploid with (AAaa) at a particular locus will produce only (AAaa) offspring (Soltis and Rieseberg 1986).

In this study, I used isozyme electrophoresis to determine the genetic structure of the four study populations of *Festuca roemeri* and to make comparisons between these populations (e.g. determine genetic distance). Isozyme data were also important for determining the breeding system of this species, enabling me to calculate whether populations are in HWE.

Although there are still many questions to be answered about outbreeding depression, it is nonetheless clear that restoration decisions based on broad theoretical generalization or unfounded assumptions about a species' biology and ecology can ultimately endanger the long-term health of that species and the ecosystem. It is for this reason that for my thesis project I am studying the breeding system and the genetic structure of *Festuca roemeri*. *F. roemeri* is an important part of the native prairie habitat of the Willamette Valley and current restoration projects at Mt. Pisgah will involve the transplanting and introduction of plants from outside the Mt. Pisgah area. Project leaders are interested in increasing the genetic diversity of the small and possibly highly inbred Mt. Pisgah population, but as of yet, the possibility of outbreeding depression has not been considered. By studying this plant's breeding system and genetic structure, I will be able to inform project leaders about whether or not they need to be concerned with this possibility and, if so, then what outside populations should be considered as possible seed sources for replanting

MATERIALS AND METHODS

Study Species

Despite the importance of *Festuca roemeri* in the restoration of the Willamette Valley, very little is known about its biology. It is a perennial and the flowers on each inflorescence are hermaphroditic. It is known to be a polyploid and is a suspected allotetrapolyploid (Barbara Wilson, personal communication, 2003). The breeding system of the plant is unknown. There are several geographically distinct populations of *F. reomeri* around Eugene located in some of the city's parks and natural areas (including Mt. Pisgah east of Eugene, Skinners's Butte, Summit Terrace in West Eugene and Rattlesnake Ridge in the Coast Range west of Junction City), but to what extent these populations are genetically distinct is also unknown. Although the genetics of the genus have been well studied and documented, I do not know of any publication that looks at this species. The lack of knowledge of this species is compounded by recent name changes and taxonomic reclassifications. Until about ten years ago, *Festuca roemeri* had been misidentified in this area as *F. rubra* (Wilson 1995). Even after this mistake was recognized, the grass was considered a variety of *F. idahoensis* and it is only recently that the identification *F. roemeri* has come to be commonly accepted (Wilson 1996).

Location and Populations

The experiments were conducted at the West Eugene Wetland Natural Area (44.02893°N, 123.17541°W), which is owned and managed by the Oregon chapter of the Nature Conservancy. The conservation area is primarily lowland and upland wet prairie and ash savanna. To conduct my studies, I used a grow-out site ("common" garden) that had been planted by the Nature Conservancy for seed harvesting. Individuals had been planted from four source populations, Rattlesnake Butte, Summit Terrace, Mt. Pisgah, and Skinners Butte, in four, side by side grid plots. The Rattlesnake source population is fairly large and is located on Rattlesnake Butte (also know as Win Ridge) just west of Junction City at the edge of the Coast Range (44.26254°N, 123.32517°W). Individuals in the Summit plot also come from a large population located at Summit Terrace in West Eugene close to the Natural Area (44.02219°N, 123.15380°W). The Pisgah population is on Mt. Pisgah in East Eugene (44.00393°N, 122.97541°W) and is a moderately sized population with individuals widely spaced and scattered. The Skinners Butte population on the southwest slope of Skinners Butte (44.05852°N, 123.09560°W) is small and closely spaced (Ed Alverson, Personal Communication) (Fig. 3).

Since the grow-out plots were not initially intended for scientific study, the exact collection and planting protocols were not recorded and thus are no longer known. However, in most cases, there was an attempt to collect seeds from a representative group across each population (Ed Alverson, Personal Communication). For the Summit population, plant divisions, rather than seeds were collected (the age of these plants, therefore, can only be approximated). In this case, the estimated center of the population was found and ten randomly selected compass directions were taken. Ten numbers between one and fifty were then selected and assigned to each compass direction, representing the meter marks where collections were to be made. These collections were made by throwing ten flags into the air and selecting divisions from a plant closest to where each flag landed. Seeds collected from at least the Rattlesnake population were put into a bag and mixed thoroughly before being selected for planting (Gil Voss, Personal Communication).

Once the seeds were collected they were sown in flats with five-inch cells (about 400 cells per flat) with an average of about 6-8 seeds per cell. The germination rates were between 50 and 100 percent. In some cases, individuals were divided to replace dead seedlings (therefore, some of the adults at the grow-out site may be clones). Once the seedlings were old enough, each was transferred to a one-inch cell and later planted in the grow-out site plots, spaced approximately every 6 inches (Gil Voss, Personal Communication). The planting grids for the Summit, Pisgah, Rattlesnake, and Skinners plots have been estimated as 19x20, 16x18, 17x65, and 16x30 individuals respectively (although, when doing the surveys it became clear that this varied from row to row since plant spacing was not exact). Planting dates for Rattlesnake, Summit, Pisgah, and Skinners plots were February 2001, Spring 2002 (date that divisions were collected), Spring 2001, and Spring 1999 respectively (Ed Alverson and Gil Voss, Personal Communications). Age of plots was estimated at two years for Mt. Pisgah, Rattlesnake, and Summit (plant divisions were assumed to be roughly one year old), and four years for Skinners Butte.

In a normal common garden experiment, individuals from each population are randomly distributed throughout the site so that variation in environment and/or soil within the site will not bias the results. This was not done at the West Eugene grow-out site but I made the assumption, for the purpose of data analysis, that the grow-out plots are fairly homogeneous. This assumption is reasonable because there was no obvious variation in soil, slope, or light at the site and the area was not large.

Population Survey

For every individual in all four populations (except for the Rattlesnake population, of which only half the individuals were surveyed) I measured survivorship (yes or no), flowering (yes or no), and the number of inflorescences per plant. Survivorship was based on evidence of dead plants and/or estimates of plant spacing. Twenty individuals were then randomly selected from each population (when the randomly selected individual was dead, I used the next surviving individual in that row) and for each individual I measured basal clump diameter (measured along the east-west axis), height of tallest leaf, height of tallest inflorescence, distance to nearest neighbor (of the same species) and pathogen damage. Pathogen damage was estimated by choosing two leaves at random (I closed my eyes and selected one leaf from the each side of the plant and one from the left side of the plant) and estimating the percentage of each leaf that was damaged by either herbivores or pathogens. By randomly selecting individuals for the detailed survey, I reduced the chance that two clones of the same individual would be surveyed.

Percent survival and survival rate data were analyzed for every individual in each population. Percent flowering and the number of inflorescences were analyzed for only living plants. From the detailed survey, all the individuals were included in the analysis of leaf height, clump diameter, distance to nearest neighbor, and percent leaf damage, while only the flowering plants were compared for inflorescence height.

To get a better idea of overall fitness for the four populations, I calculated a Standardized Fitness Estimate (SFE) similar to Montalvo (2000, 2001). The SFE for each population was calculated as (Survival Rate) x (% Flowering) x (Avg. Number of Inflorescences per Plant) x (Avg. Inflorescence Height) x (Avg. Leaf Height) x (Avg. Clump Diameter) x (% Plants w/o Leaf Damage). Each variable was first standardized by dividing by the highest population value in that category (converting each to a percent of the highest score).

Pollination Study

For the pollination study I bagged grass inflorescences with dialysis tubing. Because of time constraints, only three of the four populations, Rattlesnake, Pisgah, and Summit, were included in this portion of the study. Fifteen individuals from the Rattlesnake population were selected to act as mothers. Five inflorescences on each of these mothers were bagged before the flowers had matured and each was selected at random for one of the following treatments: (1) no pollen (only selfing possible), (2) Rattlesnake pollen (from a different individual), (3) Pisgah pollen, and (4) Summit pollen. Since there were a total of five bagged inflorescences, two of these on each plant were selected for treatment one. Individuals from the "donor" populations had also been selected to act as fathers and had been bagged before their flowers matured. These individuals were selected so that as few fathers as possible from each population were needed to pollinate all the mothers (between two and four individuals). Once the flowers had matured (anthers were visible and pollen had begun to accumulate in the bags), I cut the inflorescence head off of the father individuals, removed the bags from both the mother and the father inflorescence, and used the father inflorescence as a brush to apply pollen to the stigmas. Afterwards, I placed the FATHER bag onto the mother inflorescence. I did not remove the bags of the inflorescences marked for treatment (1). Wind was a huge problem at the study site, and not all of the bags stayed on the inflorescences. Consequently, not every mother has recorded data for every treatment.

Ideally, before the flowers were bagged, their anthers would be cut to be sure that self-pollen was not fertilizing the flower. This was not possible in this study, but since plants will usually choose outcrossed pollen over self-pollen (Richards 1997), my results were probably not significantly affected.

When the seeds had matured, the bagged inflorescences were cut and the seeds were cleaned and counted. One unbagged inflorescence was also taken from each mother to act as a control.

Isozyme Electrophoresis

Approximately twenty plants from each population were sampled and analyzed using enzyme electrophoresis. On the morning of the procedure, leaf samples were taken and stored on ice for transport back to the lab. There the leaves (approximately five one cm-long pieces) were ground on a grinding plate with four to six drops of Tris-HCl grinding buffer-PVP solution (after Soltis 1983). The extract was then soaked into Whitmann paper wicks and these were immediately loaded into the prepared potato starch gel (12% w/v hydrolyzed potato starch, after Soltis 1983). The gel was then run at a constant current of 40ma for approximately four hours and sliced and stained for PGI (Phosphoglucoisimerase). I used gel/electrode buffer system 8- after Rieseberg and Soltis (1987) and enzyme stains after Soltis et al. (1983). Several other enzymes were looked at, including PGM, GDH, AAT, 6-PGD, SkDH, TPI, IDH, ADH, and G6PDH, but these showed no significant variation or activity with the chosen buffer systems. One hour after staining, gels were scored for banding patterns.

Because *F. roemeri* is an allotetrapolyploid, interpretation of banding patterns was difficult. Genotype was determined for each individual according to a few simple rules. Whenever possible I used the simplest available interpretation. I assumed that the presence of darker bands was meaningful and that it signified an excess of that particular isozyme. Once the general genotypes had been determined, consisting of four letters, one for each allele at each of the four contributing loci, I assigned the alleles to one of the two homologous pairs of chromosomes. Following B. Wilson (Personal communication, 2003), I assumed that each allele probably only belonged to one homologous pair (the initial formation of the allotetrapolyploid was from the mating of two distantly related organisms) and that most allelic variation came from only one of the two homologous pairs (one loci must be functional and selection will limit variation at this loci). Each individual then had a four-letter genotype for PGI and this four-letter genotype was divided into two pairs of two letter genotypes, corresponding to the two pairs of homologous chromosomes.

Each pair of homologous chromosomes is inherited independently in an allopolyploid, so I was able to analyze the genetic data as if it were from two independent loci, PGI-1 and PGI-2.

Data Analysis

Except where noted, all data analysis was performed using JMP (SAS). Genetic statistics were calculated using PopGene (SAS)

RESULTS

Total Population Survey

The results of this survey are listed on Table 1. Survivorship was significantly different among the four populations (X^2=49.31, d.f.=3, P<0.0001). To take in to account the age difference, I calculated a survival rate based on the age and the percent survival (assuming that the proportion of individuals dying each year was constant). Patterns of survival closely matched patterns of survival rate, although Skinners, which had the second lowest overall percent survival, had the highest survival rate because of its age. The number of inflorescences (square root transformed to normalize residuals) also differed among populations (F=24.45, d.f.=3, P<0.0001). Pisgah and Rattlesnake were not significantly different, but both had significantly more inflorescences per plant than Summit. Skinners had significantly more inflorescences than Summit and significantly less than Rattlesnake and Pisgah. Finally, the percent flowering was significantly different among all four populations (X^2=61.84, d.f.=3, P<0.0001). It is interesting to note that the relative success of the four populations did not show a consistent pattern for the three variables (although, Pisgah and Rattlesnake, in all three cases, were more successful than Skinners and Summit). It was difficult to compare the Skinners population to the other three because of the two-year age difference.

Detailed Survey

The results of this survey are listed on Table 1 and Table 2. For the analysis, inflorescence height was log10 transformed and number of inflorescences data was, as before, square root transformed to normalize the residuals. All four primary variables, inflorescence height, number of inflorescences, leaf height, and clump diameter, were strongly correlated for all individuals (F=9.25, d.f.=9, P<0.0001) and for only flowering individuals (F=4.88, d.f.=12, P<0.0001). Inflorescence height (log10 transformed and excluding individuals without inflorescences) was significantly different between Rattlesnake, which had the highest average, and Pisgah, with the lowest average (F=6.50, d.f.=3, P=0.0009). Clump diameter was assigned as a covariate in this analysis and showed a significant effect on inflorescence height (F=6.20, d.f.=1, P=0.0164) (Table 2). Leaf height was significantly different between Rattlesnake and the other four populations and between Skinners and Summit (F=26.28, d.f.=3, P<0.0001). Clump diameter was again used as a covariate and again showed a significant effect (F=43.57, d.f.=1, P<0.0001). There was no significant difference in number of inflorescences (F=0.62, d.f.=3, P=0.6021) or for percent flowering (X^2=4.98, d.f.=3, P=0.0003), although clump diameter still was a significant covariate for both number of inflorescences (F=47.93, d.f.=1, P<0.0001) and percent flowering (F=13.14, d.f.=1, P=0.0003). Note that both these variables differed significantly in the total population survey. For clump diameter, Pisgah, Rattlesnake, and Skinners were all significantly different from Summit but not from each other (F=5.08, d.f.=3, P=0.0029). Percent leaf damage (with distance to nearest neighbor and clump diameter as covariates) did not differ significantly (F=1.6450, d.f.=3, P=0.1863). Again, the patterns for each variable were not consistent, although Rattlesnake was generally at the top and Summit was usually at the bottom.

Cumulative Fitness Indices

Standardized Fitness Estimates were highly variable among populations. Rattlesnake had the highest SFE score by far, while Summit had the lowest (Table 3). SFEs were positively correlated with geographic distance from the grow-out site ($R^2=0.8771$, P=0.0635***power issue) but were not significantly correlated with elevation ($R^2=0.0031$, P=0.9444) (Fig. 1).

Pollination Study

There was a significant difference number of seeds produced in the five treatments (F=2.58, d.f.=4, P=0.0447) (Table 4). Posthoc tests revealed, however, that only the "none" and the "self" were significantly different. There was also a significant difference in seed count between mothers (F=2.37, d.f.=14, P=0.0146). An analysis of treatment by mother effects, which included only the seven mothers for which all the treatments were still intact, was not significant (F=0.1946, d.f.=18, P=0.9923).

Isozyme Electrophoresis

After the genotypes for each individual were determined and alleles were partitioned between PGI-1 and PGI-2 (the two pairs of homologous chromosomes), I was able to calculate the number of alleles per locus, allele frequencies, number of unique alleles per locus, the percent of heterozygote individuals for each loci, and the expected number of heterozygotes assuming HWE (Table 5, 6). Expected number of heterozygotes was calculated after Levene (1949) and Nei (1978). Comparing the observed and expected number of heterozygotes at each locus for each population, I determined whether the allele frequencies departed significantly from those predicted by HWE. For each locus I also calculated a diversity index (I) based on allele richness and eveness (after Lewontin 1972) and a fixation index (F_{IS}) (after Wright 1978) based on allele frequencies at each locus.

Most of the alleles at both loci were shared by all four populations. Only one population, Summit, had any unique alleles. The allele frequencies differed significantly, however, for PGI-1 ($G^2=24.17$, d.f.=3, $P<0.0001$) as well as for PGI-2 ($G^2=35.83$, d.f.=12, $P=0.0003$). All of the populations had lower than expected percent heterozygotes and several populations differed significantly from HWE for at least one of the two loci. Although Pisgah, Rattlesnake, and Summit all departed significantly from HWE at PGI-1, only Pisgah was significantly different for both PGI-1 and PGI-2. However, at PGI-1, allele "a" was in the process of being fixed in the Pisgah population, while allele "b" was being fixed in the Rattlesnake population. The Pisgah population had the least number of alleles, the lowest percent of heterozygotes, the highest fixation index scores, and was the only population to depart significantly from HWE at both PGI-1 and PGI-2.

To compare the populations, I calculated Nei's genetic identity (I) and Nei's genetic distance (D) (Nei 1978, Crawford 1990) for all six pairs of populations (Table 7). I also compared elevation and measured geographic distance between the populations. All of the population pairs had relatively high genetic identities and relatively low genetic distances. Pisgah was most similar to the Summit population and most dissimilar to the Rattlesnake population. Genetic distance tended to increase with geographic distance. (R^2=0.0740, P=0.0740) (Fig. 2). There was no correlation between difference in elevation and genetic distance (R^2=, P=0.1429) (Fig. 3).

DISCUSSION

The results of this study have provided a much better picture of the breeding system of *Festuca reomeri*. The field study showed that this species can and does self-pollinate. Population genetic work indicated that all the populations all had a lower than expected number of heterozygotes, which may suggest that *F. roemeri* is primarily selfing. However, inbreeding due to small population sizes may also explain the deviation from HWE. The plants may prefer outcrossed pollen but may have increased homozygosity because population sizes have been so drastically reduced in the last century. The genetic data suggest that this second explanation is closer to the truth. Although a few of the populations seemed highly inbred (e.g. Pisgah), others showed allele frequencies that did not differ significantly from HWE (e.g. Skinners). Because breeding system is presumed to be the same for all four populations, variation in population size must explain the relative degrees of outcrossing and inbreeding. I suggest that this species is primarily outcrossing, with some selfing, but that habitat loss has had a significant effect on the gene pools of several populations. Even though it may be primarily outcrossing, this species seems to show no sign of reduced fitness when selfing, as evidenced by the pollination study.

The possibility that these populations are experiencing a loss of genetic diversity is especially alarming because all four seem to represent distinct subpopulations within the species. According to the data, all four populations are to a certain degree genetically and morphologically distinct. At the grow-out site, the four populations differed significantly for almost every variable that I measured. These results are most convincing when comparing the three populations of the same age, Pisgah, Rattlesnake, and Summit. When I calculated survival rate, which took into account the age difference, all four populations were also distinct. Genetically, almost every population was distinct in allele type and allele frequency. Even Pisgah and Rattlesnake, which had identical alleles for both PGI-1 and PGI-2, had very different allele frequencies and the allele being fixed at PGI-1 (i.e. the allele with the highest fixation index score) was not the same for both populations. The genetic distance calculated for Summit and Skinners and for Summit and Pisgah is consistent with other studies of local populations (Levin 1978). The genetic distance measured for the other pairs of populations, however, more closely matched numbers calculated for different species within the same genera (Levin 1978), suggesting a high degree of genetic divergence. Furthermore, there was significant variation between the four populations in the basic fitness components, survival and reproduction, at the grow-out site, suggesting that this variation is adaptive. An important next step to studying this species would be to conduct a more controlled common garden experiment (eliminating such problems as unknown and varied seed collecting and planting procedures, varying ages, and variable degrees of weed management) and to verify my isozyme electrophoresis results by examining more enzymes.

One of the most important issues addressed in this study was the possibility of outbreeding depression. Since the species is probably outcrossing and since there is significant variation between populations in the Willamette Valley, this possibility should not be ignored. The pollination study did not indicate any evidence of outbreeding effects. However, depending on the mechanism, outbreeding depression may not be apparent until the F1 or F2 generation. I recommend caution until the potential for both inbreeding depression and local adaptation are more closely examined (e.g. looking at F1 and F2 generation fitness and conducting reciprocal transplants). Pollination data also did not indicate any sign of inbreeding depression, at least for the Rattlesnake population. Seed counts for selfed inflorescences were just as high as for outcrossed inflorescences. However, inbreeding depression may not be evident until later stages in the life cycle (e.g. germination, seedling survival).

Whether or not outbreeding (or inbreeding) depression is a problem, my genetic results indicate the relationships between the four populations and suggest which individuals should be used to transplant to Mt Pisgah. According to my isozyme electrophoresis data, the Pisgah population is most similar, genetically, to the Summit population. These two populations shared the highest Nei's Identity value and the lowest Nei's Genetic Distance value. Summit was the closest population to Pisgah geographically, although the correlation between geographic distance and genetic distance was not statistically significant and many other papers have shown this relationship to be unreliable. In this study, survival at the grow-site was actually positively correlated with geographic distance. Rather than indicate a trend, this result more likely demonstrates that other factors besides geographic distance are determining environmental distance. Unfortunately, I do not have a clear idea from this study of the environmental distance between the different populations. Mt Pisgah is closest in elevation to Skinners, although elevation is only one of the many factors affecting environmental distance (and perhaps one of the least important in the relatively flat Willamette Valley). Again, it is important to consider that genetic identity and distance were calculated based only on one enzyme. To determine with more confidence which population most closely matches the Pisgah population, a more complete survey of isozyme variation should be done, as well as a reciprocal transplant experiment.

CONCLUSION

The prairie habitats of the Willamette valley are critically endangered. Studies such as this, which ask species-specific questions, are necessary for restoration efforts to succeed. This study demonstrates conclusively that at least four genetically distinct populations of *Festuca roemeri* exist and that this variation is probably adaptive. As demonstrated by this common garden study, transplanting individuals to a new site can have a drastic effect on survival and reproduction. Grow-out sites such as the one at Willow Creek are important as a seed source, but I recommend that populations should be prevented from interbreeding until a more detailed study examines the possibility of outbreeding depression in the F2 and F3 generations. Other studies should also look more closely at local adaptation and inbreeding effects in the species. Before this is done, I also recommend caution in any transplanting projects. In the case that transplants are deemed necessary, my genetic data can serve as a rough guideline for choosing the appropriate donor populations, but a more detailed isozyme study is needed to determine genetic relationships with more certainty.

This study, beyond simply examining the natural history and biology of a particular species, is important in that it highlights the complexities involved in the restoration of a species or an ecosystem. Although well intentioned, restoration projects may ultimately fail without a thorough understanding of the biology and ecology of the species involved. Since this particular ecosystem (as well as many others) is so critically endangered, it is important that the scientific community recognizes the need for more research and refocuses its energy to meet that need.

TABLES

Table 1. Population survey results. Highlighted variables are from the total population survey, while the other variables are from the detailed survey. All measurements are in centimeters. All values are population means with one standard error.

Population	N	Age (years)	%Surv*	Survival Rate (%/yr)*	%Flowr	#Infl	Infl Ht (cm)	Lf Ht (cm)	Clmp Dm (cm)	Dist NN (cm)	%Lf Dmg
Pisgah	290	2	0.71 ± 0.03	0.84	0.77 ± 0.03	28.19 ± 2.68	80.69 ± 5.27	23.10 ± 0.96	7.79 ± 0.45	24.18 ± 2.04	0.11 ± 0.02
Rattlesnake	469	2	0.78 ± 0.02	0.88	0.85 ± 0.02	18.32 ± 1.30	103.24 ± 7.45	36.78 ± 1.31	7.87 ± 0.58	15.51 ± 0.92	0.06 ± 0.02
Skinners	486	4	0.64 ± 0.02	0.89	0.60 ± 0.03	18.63 ± 1.78	97.04 ± 3.01	26.79 ± 2.08	7.56 ± 0.56	34.37 ± 3.98	0.07 ± 0.02
Summit	398	2	0.57 ± 0.02	0.75	0.65 ± 0.03	8.94 ± 1.21	87.36 ± 4.50	19.19 ± 1.49	5.01 ± 0.79	26.89 ± 2.95	0.11 ± 0.02
P-Value	NA	NA	<0.0001	NA	<0.0001	<0.0001	0.0009	<0.0001	0.0029	<0.0001	0.1863

Table 2. Details of statistical analysis with covariates.

Variable	Factor	d.f.	F Ratio	P-value
%Leaf dmg	Population	3	1.6450	0.1863
	DistNN	1	0.0073	0.9322
	Clmp Diam	1	1.2006	0.6556
Infl Ht	Population	3	6.5045	0.0009
	Clmp Diam	1	6.2011	0.0164
Lf Ht	Population	3	26.2836	<0.0001
	Clmp Diam	1	43.5661	<0.0001

Table 3. Location and comparison of four population source sites. "SFE" is the Standardized Fitness Estimate. GeogDist and ElevDist are the geographical distance and elevational difference respectively between population source site and the Willow Creek grow-out site.

Population	Source Site	Coordinates	GeogDist (miles)	Elevation (ft)	ElevDist (ft)	SFE
Pisgah	Mt Pisgah	44.00393°N, 122.97541°W	10.11	750	310	0.3484
Rattlesnake	Rattlesnake Butte	44.26254°N, 123.32517°W	17.69	1050	610	0.6011
Skinners	Skinners Butte	44.05852°N, 123.09560°W	4.45	600	160	0.3213
Summit	Summit Terrace	44.02219°N, 123.15380°W	1.19	1062.5	622.5	0.0299

Table 4. Mean seed counts for the five treatments. "Self" treatment was initially bagged, and then was not unbagged until seeds had matured. "None" treatment was never bagged.

Treatment:	Pisgah	Rattlesnake	Summit	Self	None
N:	9	12	12	27	15
Seed Count (mean ± SE):	155.78 ± 15.77	159.67 ± 14.43	155.33 ± 16.15	161.89 ± 13.10	104.00 ± 15.61

Table 5. Allele frequencies calculated from isozyme data. "TOTAL" frequencies are for all four populations pooled.

Population	Locus	A	C	E	G	I	K
Pisgah	PGI-1	0.6053	0.3947	--	--	--	--
	PGI-2	--	0.0789	0.9211	--	--	--
Rattlesnake	PGI-1	0.1667	0.8333	--	--	--	--
	PGI-2	--	0.3333	0.6667	--	--	--
Skinners	PGI-1	0.5	0.5	--	--	--	--
	PGI-2	--	0.4565	0.5217	--	--	0.0217
Summit	PGI-1	0.6538	0.3462	--	--	--	--
	PGI-2	--	0.2308	0.6154	0.0962	0.0192	0.0385
TOTAL	PGI-1	0.5	0.5	--	--	--	--
	PGI-2	--	0.2719	0.6686	0.0291	0.0058	0.0174

Table 6. Summary of isozyme electrophoresis results and genetic analysis. P-values from Likelihood ratio test for number of heterozygotes versus expected number of heterozygotes assuming HWE. A P-value less than 0.05 indicates that the population is not in HWE at that locus. Expected proportion of heterozygotes assuming HWE computed after Levene (1949) and Nei (1978).

Population	N	Locus	#Alleles	#Unique Alleles	%Hetero (Obs)	%Hetero (Exp)	HWE	Fixation (F_{IS})
Pisgah	19	PGI-1	2	0	0.0526	0.4908	**P<0.0001**	0.8899
		PGI-2	2	0	0.0526	0.1494	**P=0.0280**	0.6381
		Mean (±SD)	2	0	0.0526 ±0.0000	0.3201 ±0.2414		0.7640
Rattlesnake	18	PGI-1	2	0	0.1111	0.2857	**P=0.0168**	0.6000
		PGI-2	2	0	0.4444	0.4571	P=0.9030	0.0000
		Mean (±SD)	2	0	0.2778 ±0.2357	0.3714 ±0.1212		0.3000
Skinners	23	PGI-1	2	0	0.3913	0.5111	P=0.2489	0.2174
		PGI-2	3	0	0.4348	0.5304	P=0.5345	0.1621
		Mean (±SD)	2.5	0	0.4130 ±0.0307	0.5208 ±0.0137		0.18975
Summit	26	PGI-1	2	0	0.2308	0.4615	**P=0.0094**	0.4902
		PGI-2	5	2	0.5385	0.5679	P=0.7157	0.0332
		Mean (±SD)	3.5	1	0.3846 ±0.2176	0.5147 ±0.0752		0.2617
Total	86	PGI-1	2		0.2093	0.5029	**P<0.0001**	0.5814
		PGI-2	5		0.3837	0.4767	P<0.1881	0.1903
		Mean (±SD)	3.5		0.2965 ±0.1233	0.4898 ±0.0186		0.38585

Table 7. Genetic comparison of populations. Nei's genetic identity statistic (I) calculates the genetic similarity of two populations based on the number of shared alleles and the similarity in allele frequency. A genetic identity value of zero would indicate that the populations have no alleles in common, while an identity of one means both populations share the same alleles at equal frequencies. Nei's genetic distance (D), which is the absolute value of the natural log of I, is an estimate of "the number of allelic substitutions per locus that have occurred during the separate evolution of two populations or species." The values may range from zero (no allelic change) to infinity (Crawford 1990). GeogDist is the geographic distance between the two source populations measured in miles. ElevDist is the elevation difference between the two source populations measured in feet.

	Pisgah	**Rattlesnake**	**Skinners**	**Summit**
Pisgah	1 0 0 0	I = 0.8126 D = 0.2076 GeoDist = 24.92 miles ElevDist = 300 ft	0.8824 0.1251 7.09 150	0.9646 0.0360 8.98 312.5
Rattlesnake		1 0 0 0	0.9019 0.1033 18.11 450	0.7935 0.2314 18.65 12.5
Skinners			1 0 0 0	0.9508 0.0504 3.83 462.5
Summit				1 0 0 0

FIGURES

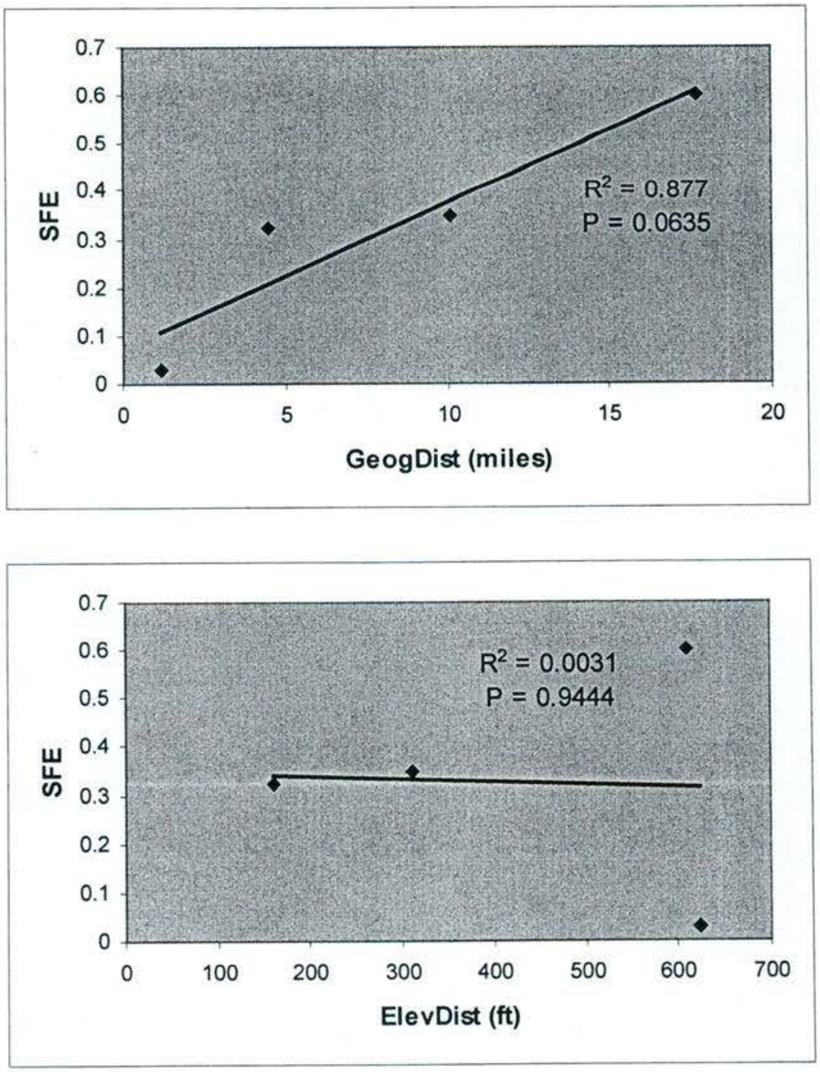

Figure 1. The Standardized Fitness Estimate for the four populations plotted against GeoDist (distance between source site and grow-out site) and ElevDist (difference in elevation between source site and grow-out site). Considering the small number of data points, the correlation between SFE and GeoDist is probably significant.

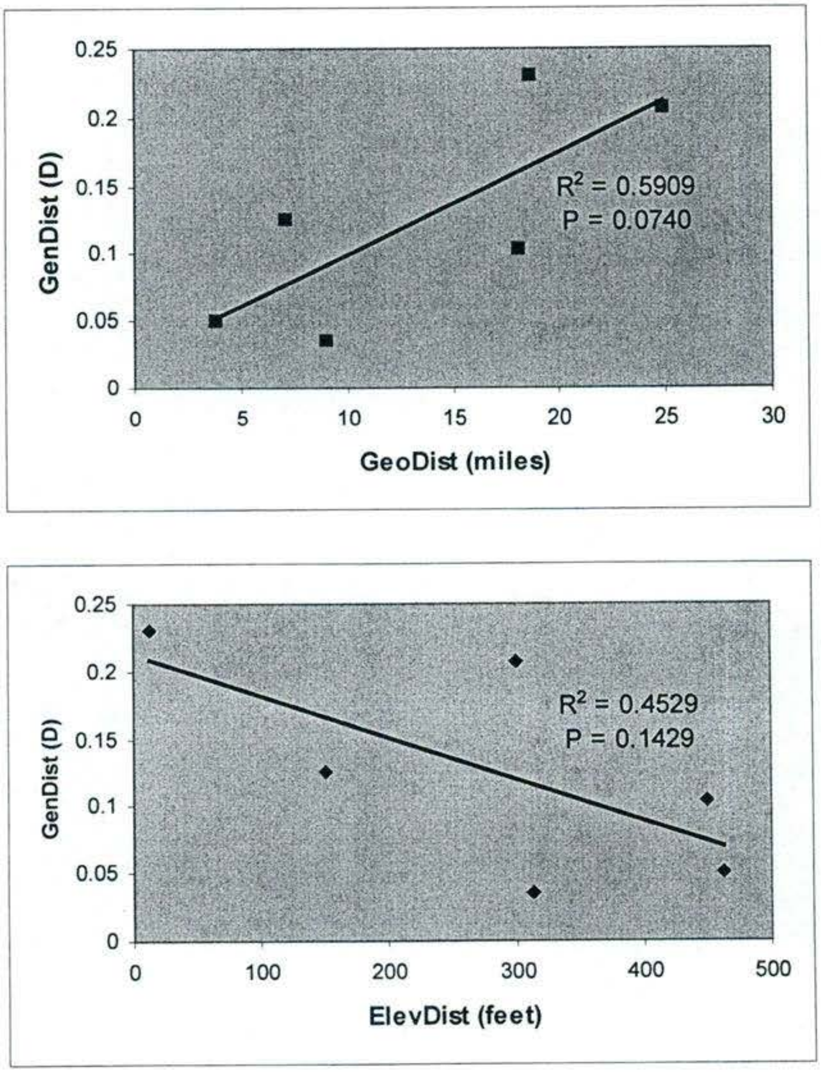

Figure 2. Nei's genetic distance for all pairs of populations plotted against ElevDist (elevation difference between two populations) and GeoDist (geographic distance between two populations). The correlation between GenDist and GeoDist was considered significant.

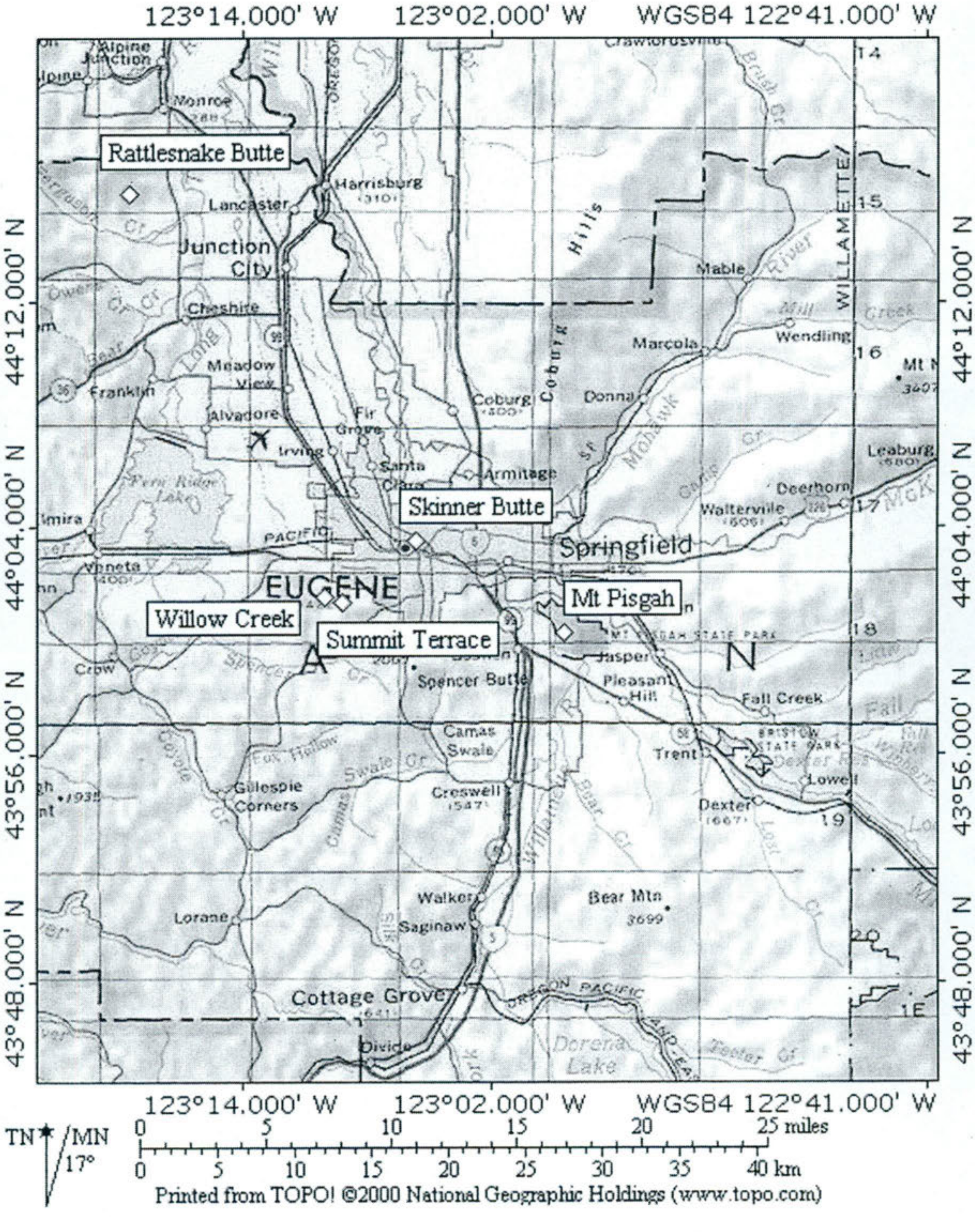

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Figure 3. Location of four population source sites as well as the West Eugene grow-out site. Map produced using TOPO!.

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